Published in final edited form as:
Am J Otolaryngol. 2012 January ; 33(1): 130–136. doi:10.1016/j.amjoto.2011.02.001.

Human Papillomavirus, Smoking, and Head and Neck Cancer

Parul Sinha, MBBS, MS[a], **Henrietta L. Logan, PHD**[a,*], and **William M. Mendenhall, MD**[b]

[a] Department of Community Dentistry and Behavioral Science, University of Florida, Gainesville, FL USA

[b] Department Of Radiation Oncology, College Of Medicine, University of Florida, Gainesville, FL USA

Abstract

Aims—Smoking and human papillomavirus (HPV) are both distinct risk factors for head and neck cancer but the nature of interaction between these two risk factors in development of head and neck cancer remains unclear The purpose of this review is to determine the potential effect of smoking in causation of HPV related head and neck carcinoma.

Method—A literature search was carried out using keywords *human papillomavirus*, *head and neck cancer*, *smoking*, *tobacco*, and *cervical cancer*. The English language articles, references and other relevant studies evaluating the association of smoking, HPV and risk of head and neck cancer were collected and analyzed.

Conclusion—Overall, our review points to smoking tobacco posing an additional risk for development of head and neck cancer in the presence of HPV infection. This is consistent with available laboratory data which show evidence of biological plausibility for interaction between smoking and progression of HPV infection to carcinogenesis. It is therefore important that cessation of smoking is promoted in smokers with HPV infection.

1. Introduction

The evidence for a link between human papillomavirus (HPV) and head and neck squamous cell carcinoma (HNSCC) has been growing ever since it was first proposed [1–4]. The literature, however, presents conflicting accounts about the nature of association between smoking and HPV in causation of HNSCC. In cervical cancer, a disease strongly associated with HPV, numerous studies point to a synergistic effect of smoking in the progression from cervical HPV infection to frank cervical cancer [5–7]. Smoking has been found to prolong the cervical HPV infection resulting in development of cervical dysplasia, progression of dysplasia towards higher grades of cervical intra-epithelial neoplasia and ultimately, invasive carcinoma [8–12]. It has also been suggested that HPV infection alone may not be sufficient for causation of cancer, and other co-factors like tobacco consumption may be required to evince its carcinogenetic effect [13–15]. Although much of our understanding about HPV in HNSCC is based on the model of cervical cancer, the degree of interaction between smoking and HPV in HNSCC is not well delineated. Some authors have observed no association [4,16–19] while other studies demonstrate an additive or synergistic

*Corresponding author. Henrietta L. Logan, UF Department of Community Dentistry and Behavioral Science, PO Box 103628, Gainesville, FL 32610-3628, USA, Telephone: (352) 273-5434, hlogan@dental.ufl.edu (H.L. Logan).

association [20–23]. Our review critically appraises the current state of the literature and seeks to determine the effect of smoking in HPV-related head and neck cancer

2. Methodology

We searched MEDLINE and Google Scholar electronic databases independently for the terms *human papillomavirus*, *head and neck cancer*, *smoking*, *tobacco*, and *cervical cancer*. We reviewed the articles available in English from 1980 through 2010 and their references. The related articles link and hand searching using key terms were utililized to locate additional relevant studies. The methodology, results, and conclusions sections of the studies were reviewed. Out of over 150 citations, we collected details on all studies which explored the association between HPV and smoking in development of HNSCC. Table 1 presents a summary of the selected studies included in our review. Based on their reported conclusions, we found the studies to be mainly of three categories: (a) studies with evidence that HPV is associated with an increased risk of HNSCC in nonsmokers, (b) studies with no difference in HPV-related HNSCC prevalence between smokers and nonsmokers, and (c) studies with evidence of an additive or synergistic effect between smoking and HPV-related HNSCC These articles and their inferences about the interaction between smoking and HPV in HNSCC are analyzed below.

3. Results

3.1. What is the evidence that HPV is associated with an increased risk of HNSCC in nonsmokers?

Several studies found an association with an elevated risk of HNSCC only in nonsmokers, and we review here the key findings from their reported results and conclusions. For instance, Snijders et al detected HPV DNA in 20.6% (n=13) of 63 patients with HNSCC and concluded smoking was not an added risk factor in the development of HNSCC [24]. It should, however, be noted that out of the HPV-positive tumors in this study, 16.7% were nonsmokers, 28.6% were heavy smokers (>20/day), and 50% were former smokers [24]. Thus, a large proportion of HPV-positive cases had exposure to tobacco as well. Fouret et al observed a 50% prevalence of HPV infection in nonsmokers (n=5 out of 10 nonsmokers; 95%CI = 1.9–8.1) as compared to 8.5% prevalence in smokers (n=15 out of 177 smokers; 95%CI = 0.5–1.4) [16]. The number of nonsmokers in this study was considerably small. Koch et al noted a twofold higher rate of HPV-associated tumors in non-current smokers in comparison to current smokers (OR = 2.2; 95% CI: 1.0– 4.7; p = .05) [25]. The p value of this study is only marginally significant and the group of non-current smokers included both never smokers and former smokers.

A hospital-based case-control study examining the risk factor profiles for 240 cases with HNSCC and stratified by tumor HPV-16 status as determined by in-situ hybridization found that neither smoking status (P_{trend} = 0.40) nor increasing intensity (P_{trend} = 0.27) or increasing duration (P_{trend} = 0.55) increased the odds of HPV-positive HNSCC [26]. However, very heavy daily use of tobacco (> 2 packs or 40 cigarettes) showed a trend towards increased odds of HPV-positive HNSCC (OR = 3.2, 95% C.I. = 0.73 – 13.9) [26]. Another study conducted by these authors evaluated the interaction between tobacco and exposure to HPV as assessed by detection of serum antibodies to the HPV-16 L1 protein. They found no evidence of synergy between exposure to HPV and smoking [17]. Both HPV L1 seropositivity (OR = 27.8, 95% C.I.= 6.7 – 114.6, p=0.12) and oral HPV infection (OR = 13.2, 95% C.I.= 2.4 = 65.8, p=0.29) were highly associated with oropharyngeal cancer among patients with a history of heavy tobacco use (>20 pack-years), however, the association was greater among patients without such a history (OR for seropositivity = 37.1, 95% C.I.= 15.6 – 88.4; OR for oral HPV16 infection = 17.2, 95% C.I.= 6.4 – 46.3). HPV 16

seropositivity and risk of HNSCC was also analyzed by Applebauam et al. [19]. They observed that, among never smokers, the risk of pharyngeal cancer increased approximately 30 fold for the HPV16-seropositive subjects compared with the seronegative subjects (OR = 32.5, 95% CI = 13.3 to 79.5). However, among the HPV16-seropositive subjects, smoking cigarettes did not increase risk of pharyngeal cancer [19]. A wide confidence interval of the measured association of risk is obvious in these three above mentioned studies.

In addition to the authors finding an association of HPV and HNSCC only in nonsmokers, there are researchers who observed no difference by smoking status in HPV-positive and HPV-negative tumors [4,27–30]. Paz et al compared the clinical behavior of HPV-positive and HPV-negative HNSCC but found no statistical association between HPV status of tumors and tobacco use [27]. In high risk disease sites like the oropharynx, the proportion of tobacco users in the HPV-positive cases was 81% as compared to 85.7% in HPV negative cases [27]. In a study by Gillison et al, smokers were more frequent than nonsmokers in the HPV-negative HNSCC group (67% vs. 63%), whereas nonsmokers were more frequent than smokers in the HPV-positive oropharynx group (15% vs. 13%). These differences were not statistically significant [26].

3.2. What is the evidence that smoking increases the risk of HPV-associated HNSCC?

Our review brings to the forefront several studies which have found an additive or synergistic interaction between smoking and an increased risk of HNSCC in HPV-infected individuals. One of these studies comes from a large multicenter group which investigated the interaction between smoking and HPV serology status of patients with HNSCC. They found the risk to be additive for smoking and HPV E6/E7 seropositivity [20]. When compared with never smokers who were negative for HPV16 E6 and E7, smokers who were negative for HPV16 E6 and E7 (OR= 11.2, 95% CI= 5.9 to 21.4), never smokers who were positive for HPV16 E6 or E7 (OR= 64.5, 95% CI= 18.3 to 226.7), and smokers who were positive for HPV16 E6 or E7 (OR= 56.2, 95% CI= 22.5 to 140.4) had an increased risk for cancer of the oropharynx [20]. This association showed a pattern typical of an additive effect between the two risk factors: smoking and HPV.

An additive interaction was observed in another study in which HPV positive smokers had a greater risk of head and neck cancer (OR = 5.5, 95% CI=2.1–14.1) than individuals who used tobacco (OR= 1.6, 95% C.I. = 0.9–2.8) or were detected with HPV (OR= 1.4, 95% C.I. = 0.5–3.6) alone [23]. HPV was detected in oral exfoliated cells and a hemocytometer was used to ensure an adequate number of cells for each Polymerase Chain Reaction (PCR) of every sample to detect HPV DNA. In order to reduce the potential misclassification bias associated with carcinogenic effects from exposure to tobacco, the analysis was restricted to the group who never used tobacco, excluding both low and former users. In a later study of 201 incident cases of HNSCC by the same group of authors, OR for heavy smoking (>30 pack-years) and HPV VLP (Virus like Protein) seropositivity was found to be 2.3(95% C.I=1.1–4.8) while for heavy smoking and HPV sereonegativity, it was 2.6 (95% C.I. =1.4–5.0). The authors therefore concluded that tobacco smoking significantly increased the risk of cancer in HPV-seropositive individuals and that HPV infection may not be sufficient but requires the accumulation of additional cellular changes to cause HNSCC [22].

A population-based case control study on 284 patients with oral and oropharyngeal squamous cell carcinoma demonstrated a synergistic interaction between smoking and HPV serology in increasing the risk of HNSCC [21]. The joint association of cigarette smoking and HPV-16 seropositivity (OR = 8.5; 95% CI = 5.1–14.4) was much stronger than predicted from the sum of individual associations with smoking (OR = 3.2; 95% CI = 2.0–5.2) and HPV-16 seropositivity (OR = 1.7; 95% CI = 1.1–2.6). This study used a sex and

age matched control and detected the presence of HPV by PCR of tissue samples as well as Enzyme Linked Immunosorbent Assay (ELISA) for detection of antibodies to L1 [21].

Along with a positive interaction towards causation of HNSCC, tobacco smoking has showed a strong statistical trend for an adverse effect on disease specific survival [31–33]. An increase in distant metastases and tumor recurrence among HPV-positive patients was observed in a study by Maxwell et al [32]. Heavy smoking of more than 20 pack-years has been reported by Gillison et al to be associated with an increased hazard ratio of death (HR= 1.79) in HPV-positive oropharynx cancer patients relative to HPV-positive oropharyngeal cancer with <20 pack-years [33].

4. Discussion

Overall, the putative evidence from the above review points to smoking posing an additional risk for development of HNSCC in presence of HPV infection. Where counter examples of such an association exist, the studies are limited by small sample sizes [16,24], weak statistical evidence, [16,17,24–26] and inconsistent definitions of smoking status [25]. Criteria of light and heavy smoking, as well as the definition of current, never, and former smokers, are not uniform. The dose and duration of tobacco exposure in most of the studies is self-reported. The strength of the reported conclusions would have been greater if the exposure had been confirmed with cotinine or other bioassays. On the other hand, the studies showing an additive or synergistic interaction between smoking and HPV have larger sample sizes and adequate controls [20–23] which support greater generalizability of these results to other populations.

4.1. Is it biologically plausible that smoking can promote development of HPV-related HNSCC?

A positive association between smoking and HPV toward causation of HNSCC appears to be biologically plausible based on our review of clinical studies and supporting evidence of pathological interactions between the two risk factors. HPV may have evolved mechanisms to escape immune-surveillance but the transient nature of infection lends little credence to its ability to cause neoplastic changes in the absence of any contributory factors [11,14,22,34]. In HPV infection of head and neck particularly, the viral copy number has not been found to be substantially increased, suggesting that alternate pathways mediated by co-carcinogens like tobacco may be involved in HNSCC related to HPV [35].

Tobacco has been potentially linked with all major phases of HPV-related carcinogenesis: initiation, promotion, and progression. Histopathologically, smoking causes cellular and structural alterations in tonsils, leading to an increased oral acquisition of HPV [36]. This has been corroborated clinically as researchers have observed a high prevalence of HPV infection in smokers, particularly current smokers [37]. Smoking is also known to suppress the mediators of immune function thus facilitating persistence of HPV infection—a step crucial to development of HPV-related cancer [8,11]. Inactivation of the tumor suppressor gene p53 by the HPV E6 oncoprotein is an important step in causation of HPV related malignancy at the molecular level [19,20,26,38]. The DNA damage caused by smoking may further impair the cell's ability to recuperate from the mutagenic insults along with an increase in frequency of p53 mutations [39,40]. It has also been suggested that the carcinogenetic potential of HPV increases with viral integration with host DNA, an effect resulting in overexpression of HPV oncogenes. The process of integration occurs at fragile sites or 'hot spots' of DNA breakage and there is evidence that tobacco smoking induces DNA breaks in human cells [41–43]. Thus, an increased frequency of HPV integration in smokers may increase the risk of carcinogenesis in the presence of HPV infection. Moreover, laboratory research has found current smokers to have statistically significantly

greater viral loads than never smokers, thus implying that cessation may result in attenuation of the viral load [44].

Tobacco-associated genetic or epigenetic alterations have also been postulated to result in acceleration of the disease progression in HPV infected individuals [31,32,45]. This is supported by clinical and pathological evidence of a poorer survival of HPV-positive HNSCC patients in smokers as compared with HPV-positive patients with nonsmokers [31–33,45,46].

4.2. Can individual susceptibilities influence measures of association between HPV and tobacco exposure for development of HNSCC in different studies?

As the current paradigm is shifting towards understanding the molecular progression of HPV-related head and neck tumors, it is becoming recognized that individual susceptibilities may explain the variation in relationship between smoking and HPV. Variants of highly polymorphic but critical tumor-suppressor genes like p53 and p73 interact with the HPV oncoproteins E6 and E7 and result in a much higher risk of HPV-16 associated oral cancer in nonsmokers than ever smokers [47–49]. The frequency of the high-risk polymorphisms among cases in one of the studies was 40 – 44% [48]. Though a detailed discussion of these individual variations is beyond the scope of our review, it would be a relevant factor for future studies on the interaction between HPV and smoking.

5. Conclusion

Our review of the existing literature on the association between smoking and HPV in causation of HNSCC identifies smoking to have the potential to promote infection, persistence, and the carcinogenetic effect of HPV. Though prospective studies on the natural history would better unravel the possible interactions, on the basis of the current laboratory and clinical studies, we conclude that the HPV-related tumors should not be considered as an occurrence exclusive to nonsmokers. Thus, along with close surveillance for early detection of HNSCC, early cessation of smoking should be considered imperative in smokers, particularly heavy smokers with HPV infection. Current evidence indicates that stop smoking efforts can lead to a reduction in the viral loads and slow the progression to HPV-related malignancy. Cessation is *sine qua non* also in view of evidence of a poorer survival status and increased risk of recurrence in smokers with HPV-positive HNSCC as compared to nonsmokers.

Acknowledgments

Support from NIDCR, grant 1U54DE019261.

References

1. Syrjanen K, Syrjanen S, Lamberg M, et al. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous-cell carcinogenesis. Int J Oral Surg. 1983; 12:418–24. [PubMed: 6325356]
2. Smith EM, Hoffman HT, Summersgill KS, et al. Human papillomavirus and risk of oral cancer. Laryngoscope. 1998; 108:1098–103. [PubMed: 9665264]
3. Franceschi S, Munoz N, Snijders PJF. How strong and how wide is the link between HPV and oropharyngeal cancer? Lancet. 2000; 356:871–2. [PubMed: 11036888]
4. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000; 92:709–20. [PubMed: 10793107]

5. Daling JR, Madeleine MM, McKnight B, et al. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiology Biomarkers & Prevention. 1996; 5:541–8.
6. Kjaer SK, Engholm G, Dahl C, et al. Case-control study of risk factors for cervical squamous cell neoplasia in Denmark. 4. Role of smoking habits. Eur J Cancer Prev. 1996; 5:359–65. [PubMed: 8972255]
7. Burger MPM, Hollema H, Gouw ASH, et al. Cigarette-smoking and human papillomavirus in patients with reported cervical cytological abnormality. Br Med J. 1993; 306:749–52. [PubMed: 8387842]
8. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis: role of parity, oral contraceptives, and tobacco smoking. Journal of the National Cancer Institute Monographs. 2003:20–8. [PubMed: 12807941]
9. Prokopczyk B, Cox JE, Hoffmann D, et al. Identification of tobacco-specific carcinogen in the cervical mucus of smokers and nonsmokers. J Natl Cancer Inst. 1997; 89:868–73. [PubMed: 9196253]
10. Ho GYF, Kadish AS, Burk RD, et al. HPV 16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia. Int J Cancer. 1998; 78:281–5. [PubMed: 9766558]
11. Giuliano AR, Sedjo RL, Roe DJ, et al. Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). Cancer Causes Control. 2002; 13:839–46. [PubMed: 12462549]
12. Castle PE, Wacholder S, Lorincz AT, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J Natl Cancer Inst. 2002; 94:1406–14. [PubMed: 12237286]
13. Winkelstein W. Smoking and cervical-cancer - current status: a review. Am J Epidemiol. 1990; 131:945–57. [PubMed: 2188500]
14. Kim MS, Shin KH, Baek JH, et al. HPV-16, Tobacco-specific N-Nitrosamine, and N-Methyl-N′-nitro-N-nitrosoguanidine in oral carcinogenesis. Cancer Res. 1993; 53:4811–6. [PubMed: 8402666]
15. Li SL, Kim MS, Cherrick HM, et al. Sequential combined tumorigenic effect of HPV-16 and chemical carcinogens. Carcinogenesis. 1992; 13:1981–7. [PubMed: 1330348]
16. Fouret P, Monceaux G, Temam S, et al. Human papillomavirus in head and neck squamous cell carcinomas in nonsmokers. Archives of Otolaryngology-Head & Neck Surgery. 1997; 123:513–6. [PubMed: 9158399]
17. D'Souza G, Kreimer AR, Viscidi R, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007; 356:1944–56. [PubMed: 17494927]
18. Furniss CS, McClean MD, Smith JF, et al. Human papillomavirus 6 seropositivity is associated with risk of head and neck squamous cell carcinoma, independent of tobacco and alcohol use. Ann Oncol. 2009; 20:534–41. [PubMed: 19087986]
19. Applebaum KM, Furniss CS, Zeka A, et al. Lack of association of alcohol and tobacco with HPV16-associated head and neck cancer. J Natl Cancer Inst. 2007; 99:1801–10. [PubMed: 18042931]
20. Herrero R, Castellsague X, Pawlita M, et al. Human papillomavirus and oral cancer: The international agency for research on cancer multicenter study. J Natl Cancer Inst. 2003; 95:1772–83. [PubMed: 14652239]
21. Schwartz SM, Daling JR, Doody DR, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst. 1998; 90:1626–36. [PubMed: 9811312]
22. Smith EM, Rubenstein LM, Haugen TH, et al. Tobacco and alcohol use increases the risk of both HPV-associated and HPV-independent head and neck cancers. Cancer Causes Control. 2010
23. Smith EM, Ritchie JM, Summersgill KF, et al. Human papillomavirus in oral exfoliated cells and risk of head and neck cancer. J Natl Cancer Inst. 2004; 96:449–55. [PubMed: 15026470]
24. Snijders PJF, Scholes AGM, Hart CA, et al. Prevalence of mucosotropic human papillomaviruses in squamous-cell carcinomas of the head and neck. Int J Cancer. 1996; 66:464–9. [PubMed: 8635861]

25. Koch WM, Lango M, Sewell D, et al. Head and neck cancer in nonsmokers: A distinct clinical and molecular entity. Laryngoscope. 1999; 109:1544–51. [PubMed: 10522920]
26. Gillison ML, D'Souza G, Westra W, et al. 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; 100:407–20. [PubMed: 18334711]
27. Paz IB, Cook N, OdomMaryon T, et al. Human papillomavirus (HPV) in head and neck cancer - An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. Cancer. 1997; 79:595–604. [PubMed: 9028373]
28. Park NH, Gujuluva CN, Baek JH, et al. Combined oral carcinogenicity of HPV-16 and benzo(a)pyrene - an in-vitro multistep carcinogenesis model. Oncogene. 1995; 10:2145–53. [PubMed: 7784058]
29. Ha PK, Pai SI, Westra WH, et al. Real-time quantitative PCR demonstrates low prevalence of human papillomavirus type 16 in premalignant and malignant lesions of the oral cavity. Clin Cancer Res. 2002; 8:1203–9. [PubMed: 12006539]
30. Sisk EA, Soltys SG, Zhu SB, et al. Human papillomavirus and p53 mutational status as prognostic factors in head and neck carcinoma. Head and Neck-Journal for the Sciences and Specialties of the Head and Neck. 2002; 24:841–9.
31. Hafkamp HC, Manni JJ, Haesvoets A, et al. Marked differences in survival rate between smokers and nonsmokers with HPV 16-associated tonsillar carcinomas. Int J Cancer. 2008; 122:2656–64. [PubMed: 18360824]
32. Maxwell J, Kumar B, Feng F, et al. Tobacco use in human papillomavirus-positive advanced oropharynx cancer patients related to increased risk of distant metastases and tumor recurrence. Clin Cancer Res. 2010:16.
33. Gillison ML, Harris J, Westra W, et al. Survival outcomes by tumor human papillomavirus (HPV) status in stage III–IV oropharyngeal cancer (OPC) in RTOG 0129. J Clin Oncol. 2009:27.
34. Kanodia S, Fahey LM, Kast WM. Mechanisms used by human papillomaviruses to escape the host immune response. Curr Cancer Drug Targets. 2007; 7:79–89. [PubMed: 17305480]
35. Ostwald C, Muller P, Barten M, et al. Human papillomavirus DNA in oral squamous-cell carcinomas and normal mucosa. J Oral Pathol Med. 1994; 23:220–5. [PubMed: 8046660]
36. Torre V, Bucolo S, Giordano C, et al. Palatine tonsils in smoker and non-smoker patients: a pilot clinicopathological and ultrastructural study. J Oral Pathol Med. 2005; 34:390–6. [PubMed: 16011606]
37. D'Souza G, Agrawal Y, Halpern J, et al. Oral sexual behaviors associated with prevalent oral human papillomavirus infection. J Infect Dis. 2009; 199:1263–9. [PubMed: 19320589]
38. Portugal LG, Goldenberg JD, Wenig BL, et al. Human papillomavirus expression and p53 gene mutations in squamous cell carcinoma. Archives of Otolaryngology-Head & Neck Surgery. 1997; 123:1230. [PubMed: 9366703]
39. Moore TO, Moore AY, Carrasco D, et al. Human papillomavirus, smoking, and cancer. J Cutan Med Surg. 2001; 5:323–8. [PubMed: 11907844]
40. Wei LL, Gravitt PE, Song HB, et al. Nitric Oxide Induces Early Viral Transcription Coincident with Increased DNA Damage and Mutation Rates in Human Papillomavirus-Infected Cells. Cancer Res. 2009; 69:4878–84. [PubMed: 19487298]
41. Nakayama T, Kaneko M, Kodama M, et al. Cigarette-smoke induces DNA single-strand breaks in human-cells. Nature. 1985; 314:462–4. [PubMed: 2984577]
42. Luo LZ, Werner KM, Gollin SM, et al. Cigarette smoke induces anaphase bridges and genomic imbalances in normal cells. Mutation Research-Fundamental and Molecular Mechanisms of Mutagenesis. 2004; 554:375–85. [PubMed: 15450433]
43. Ragin CCR, Modugno F, Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res. 2007; 86:104–14. [PubMed: 17251508]
44. Xi LF, Koutsky LA, Castle PE, et al. Relationship between cigarette smoking and human papilloma virus types 16 and 18 DNA load. Cancer Epidemiology Biomarkers & Prevention. 2009; 18:3490–6.

45. Ghosh A, Ghosh S, Maiti GP, et al. SH3GL2 and CDKN2A/2B loci are independently altered in early dysplastic lesions of head and neck: correlation with HPV infection and tobacco habit. J Pathol. 2009; 217:408–19. [PubMed: 19023882]

46. Weinberger PM, Yu ZW, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus-associated oropharyngeal cancers with favorable prognosis. J Clin Oncol. 2006; 24:736–47. [PubMed: 16401683]

47. Ji XM, Neumann AS, Sturgis EM, et al. p53 codon 72 polymorphism associated with risk of human papillomavirus-associated squamous cell carcinoma of the oropharynx in never-smokers. Carcinogenesis. 2008; 29:875–9. [PubMed: 18258602]

48. Chen XM, Sturgis EM, El-Naggar AK, et al. Combined effects of the p53 codon 72 and p73 G4C14-to-A4T14 polymorphisms on the risk of HPV16-associated oral cancer in never-smokers. Carcinogenesis. 2008; 29:2120–5. [PubMed: 18701437]

49. Chen XM, Sturgis EM, Etzel CJ, et al. p73 G4C14-to-A4T14 Polymorphism and Risk of Human Papillomavirus-associated Squamous Cell Carcinoma of the Oropharynx in Never Smokers and Never Drinkers. Cancer. 2008; 113:3307–14. [PubMed: 18988287]

Table 1

Details of selected studies on association between smoking and HPV in HNSCC

Authors	Source of subjects & design	Cases	Number of Cases/Controls	HPV assay method correlated with smoking status	Relation between frequency of smoking and HPV status in cases (%)	Odds Ratio for HNSCC (positive HPV infection & smoking)	Conclusion about interaction between smoking and HPV
Snijders, 1996 [24]	Hospital based prevalence study	Newly diagnosed HNSCC patients sampled during surgical treatment	63/0	PCR of snap frozen tumor tissue and Southern Blot analysis for multiple low and high risk HPV types	Of HPV positive cases, 16.7% were nonsmokers, 28.6% were heavy smokers (>20/d) and 50% were former smokers	NR	No correlation between HPV and smoking status
Paz, 1997 [27]	Hospital based prevalence study	Treated patients with head and neck cancers	167/0	PCR of snap frozen tumor tissue for HPV 6, 16, 18 and Southern Blot hybridization	81% smokers in HPV positive cases vs. 85.7% in HPV negative cases (Difference NS)	NR	No difference in HPV positive HNSCC prevalence in smokers & nonsmokers
Fouret, 1997 [16]	Hospital based prevalence study	Treated patients with oropharyngeal, hypopharyngeal, laryngeal cancer	187/0	PCR & sequencing of paraffin embedded samples using E6-directed primers	HPV positivity in 8.5% of smokers (95% CI = 0.5–1.4) vs. 50% of nonsmokers (95% CI = 1.9–8.1) (P=0.003)	NR	HPV may play a role in HNSCC in nonsmokers.
Schwartz, 1998 [21]	Population based case-control study	Newly diagnosed oral & oropharyngeal cases including treated cases	284/477	PCR & sequencing of tumor & exfoliated cells (brush) for high and low risk HPV types; ELISA for HPV L1	31.3% current smokers in HPV L1 seropositive cases vs. 20.1% in seronegative cases	8.5 (5.1–14.4) for smokers and HPV 16 L1 seropositivity	Synergistic association of smoking and HPV 16 L1 seropositivity (Synergy Index = 2.6 (1.3–5.0)
Gillison, 2000 [4]	Hospital based retrospective study	Newly diagnosed or recurrent cases of HNSCC	253/0	PCR, Southern Blot and in situ hybridization for high and low risk HPV types	67% smokers in the HPV-negative vs. 63% HPV positive cases (Difference NS)	NR	No difference in HPV positive HNSCC prevalence in smokers & nonsmokers
Herrero, 2003 [20]	Hospital based multicenter case control study	Newly diagnosed pt with oral & oropharyngeal before treatment	1670/1732	PCR of tumor tissue & exfoliated cells by brushing; ELISA for antibodies to L1, E6, E7	Among 29 oropharyngeal cancer cases with HPV E6/E7 positivity, 24% nonsmokers and 76% smokers	56.2 (22.5 to 140.4) for smokers who were positive for HPV16 E6 or E7 (no stratification by dose-duration)	Additive effect for HPV E6/E7 seropositivity and tobacco smoking or chewing
Smith, 2004 [23]	Hospital based case-control study	Newly diagnosed pt with oral &	201/333	PCR & sequencing of oral exfoliated	13% heavy smokers (>30 pack-	5.5 (2.1–14.1)	Additive effect between tobacco

Authors	Source of subjects & design	Cases	Number of Cases/Controls	HPV assay method correlated with smoking status	Relation between frequency of smoking and HPV status in cases (%)	Odds Ratio for HNSCC (positive HPV infection & smoking)	Conclusion about interaction between smoking and HPV
		oropharyngeal cases before treatment		cells (rinse) for both HR and LR types	years) in HPV-HR cases vs. 44% in HPV negative cases		use and detection of HPV-DNA
D'Souza, 2007 [17]	Hospital based case-control study	Newly diagnosed oropharyngeal cancer cases before treatment	100/200	HPV-16 in situ hybridization, DNA analysis of both HR and LR types, ELISA for antibodies against L1	NR	27.8 (6.7–114.6) for HPV-16 L1 seropositivity and heavy smoking; 37.1 (15.6–88.4) for moderate smoker (<20) & 2.8 (1.2–6.4) for heavy smokers and seronegative cases	No additive/ synergistic effect of tobacco with HPV (Synergy Index 0.7 =0.5–1.1)
Applebaum 2007 [19]	Case control study	Incident cases of HNSCC both before and after treatment	485/549	HPV competitive Luminex Immunoassay for antibodies to HPV 16 L1	In seropositive vs. seronegative cases, Never smokers (15% vs. 5%); 0–20 pack yr (11% vs. 7%); 20– <45 (11% vs. 15%); > 45 (12% vs. 24%)	HPV 16 seropositivity a/w 30-fold increase risk of pharynx ca among never smokers (OR = 32.5, 95% CI = 13.3 to 79.5)	Tobacco use does not increase the risk of HPV 16 – associated pharyngeal cancer.
Gillison, 2008 [26]	Hospital based case-control study	Newly diagnosed pt with HNSCC before treatment	240/322	In situ hybridization system for HPV-16 DNA detection in paraffin embedded tumor tissue for HR and LR types	52% never smokers; 29% former smokers; 18% current smokers among HPV 16-positive cases	very heavy daily users of tobacco (>40/d) had non statistically significantly increased odds of HPV-16 DNA positive HNSCC (3.2, 95% CI = 0.73 to 13.9)	No additive/ synergistic effect, positive trend
Furniss, 2009 [18]	Case control study	Incident cases of HNSCC both before and after treatment	486/548	Competitive Luminex Immunoassay for HPV 6,11,16,18	NR	For pharyngeal cancer cases with heavy smoking, OR= 3.1 (2.0–4.8) among HPV6-seronegative and 1.6 (0.7–3.5) in HPV6-seropositive cases	HPV6 seropositivity independently associated with increased risk for pharyngeal tumors and is not associated with smoking
Smith, 2010 [22]	Hospital based case-control study	Newly diagnosed HNSCC cases	201/324	ELISA for antibodies to VLP and PCR of tumor tissue	22% heavy smokers >30 pack years) in HPV VLP seropositive cases vs. 26% heavy smokers in seronegative cases	OR for heavy smoking (>30 pack-years) and HPV VLP seropositivity=2.3(1.1–4.8); for sereonegativity, OR=2.6 (1.4–5.0)	Tobacco use increases risk of HNSCC in both HPV seropositive and seronegative patients

NR= Not reported; NS= Not significant; HR= High risk HPV type; LR= Low risk HPV type; VLP=Virus like proteins

Am J Otolaryngol. Author manuscript; available in PMC 2013 January 1.