Mucosal and cutaneous human papillomaviruses in head and neck squamous cell papillomas

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ABSTRACT: *Background.* Conflicting data exist regarding the contribution of human papillomavirus (HPV) to the development of head and neck squamous cell papillomas.

Methods. Formalin-fixed paraffin-embedded papillomas were tested for 28 mucosal and 79 cutaneous HPVs using polymerase chain reaction (PCR)-based methods.

Results. Eighty-three papillomas (43 oropharyngeal, 31 oral, 6 laryngeal, and 3 nasopharyngeal) were analyzed. Twenty-four samples (28.9%) harbored mucosal HPVs: 3 oropharyngeal (6.9%), 15 oral (48.3%), 4 laryngeal (66.7%), and 2 nasopharyngeal papillomas (66.7%). Eighty-one cases were also tested for cutaneous HPVs, detected in 16 lesions (19.7%): 11 (13.5%) harbored only cutaneous types, and 5 (6.2%) were positive for both cutaneous and mucosal HPVs. Among these 81 cases, prevalence of mucosal and/or cutaneous HPV infection was 43.2%.

Conclusion. HPV DNA detection in a fraction of head and neck papillomas supports the role of HPV in their development. However, other markers need to be considered to confirm the association of HPV infection with these lesions. © 2016 Wiley Periodicals, Inc. *Head Neck* **39:** 254–259, 2017

KEY WORDS: oral cavity, oropharynx, papilloma, papillomavirus, prevalence

INTRODUCTION

Benign lesions of the mucosal surfaces of the head and neck region are quite common. They more often develop in the oral cavity, but may also arise in the oropharynx, larynx, and nasopharynx. They appear as exophytic, hyperplastic lesions, which are sessile or pedunculated, and have a smooth, verrucous, or cauliflower-like morphology. A very common form of these benign lesions is represented by papillomas. Unfortunately, there is much confusion concerning the use of this term and the categorization of these lesions, which may include entities that are often clinically indistinguishable.

The Pathology and Genetics of Head and Neck Tumors of the 2005 World Health Organization (WHO) Classification of Tumors defines papillomas as "a range of localized hyperplastic exophytic and polypoid lesions of hyperplastic epithelium with a verrucous or cauliflower-like morphology."[1] The "Digital manual for the early diagnosis of oral neoplasia" of the International Agency for Research on Cancer (IARC) defines a papilloma as "a benign painless exophytic growth with a narrow stalk and containing numerous finger-like projections."[2] However, although the WHO considers the oral counterpart of the common warts (verruca vulgaris) as a squamous papilloma and the oral counterpart of the anogenital warts (condyloma acuminatum) as a distinct type of lesion, the IARC digital manual specifies that oral papillomas enter into the differential diagnosis with the oral verruca vulgaris and the oral condyloma acuminatum. This definition implies that these lesions should be considered as 3 different diagnostic categories.

The WHO classification specifies that squamous papillomas, including the oral verruca vulgaris, are usually solitary, whereas oral condylomas tend to appear as multiple, clustered lesions, larger in size than the former. Additionally, oral condylomas may recur after excision/ablation, whereas recurrence is rare for papillomas and oral verruca vulgaris. However, all these benign lesions may display minimal histological differences, making the differential diagnosis challenging.

In addition to the ambiguity regarding the diagnostic categorization of papillomas, their etiology is also problematic. In fact, it is commonplace to assume that head and neck papillomas are caused by human papillomavirus (HPV) infection.[3,4] HPVs are species-specific viruses, which show strict tropism for the stratified squamous epithelium. Over 170 types have been identified so far that can infect the skin

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or the mucosal surface of the aerodigestive and anogenital tract.[5] Based on their tropism, they have been classically distinguished in cutaneous and mucosal types. The former group is involved in the development of benign skin conditions (eg, common and plantar warts). Differently, mucosal HPVs, which can be distinguished as low-risk and high-risk types, may cause benign anogenital warts (low-risk types) and premalignant and malignant lesions (high-risk types). High-risk HPVs cause the majority of cervical and anal cancers, in addition to a subset of vulvar, vaginal, penile, and head and neck cancers.[6] In the head and neck region, they are mainly responsible for the development of oropharyngeal squamous cell carcinomas (SCCs).[7]

HPV presence is generally reported in less than half of head and neck papillomas. The WHO defines the oral verruca vulgaris as a squamous papilloma associated with HPV infection. In fact, these lesions are usually caused by cutaneous HPV types, such as HPV2 and HPV4, and may develop because of autoinoculation or horizontal transmission. Instead, the oral condyloma acuminatum often harbors mucosal HPVs, such as HPV6 and HPV11. HPV prevalence may reach 100% in these lesions.[1]

HPV testing is not utilized in the routine diagnostic process of head and neck papillomas, but, sometimes, the histological features may not be sufficiently distinctive for a correct classification. In these cases, it might be useful to investigate the possible viral origin of the lesion through a nonsubjective method, such as HPV testing, especially considering that certain types of head and neck lesions, such as oral condylomas, have a high risk of recurrence.

A large number of HPV types are included in the beta and gamma genera of the HPV phylogenetic group, but their direct involvement in human diseases is poorly investigated. Beta HPVs, which are considered to be cutaneous types being largely detected in the skin, appear to cooperate with ultraviolet irradiation in the development of nonmelanoma skin cancer.[8,9] However, recent data have shown that they are also present in the oral mucosa.[10,11]

In the current study, we performed a thorough biomolecular investigation of HPV infection in a series of head and neck lesions with a clinical diagnosis of papilloma and a histological diagnosis of squamous cell papilloma. The presence of 107 different HPV types of the alpha, beta, gamma, and mu genera were assessed. Our results provide useful information on the possible nature and etiology of lesions that are categorized as squamous cell papillomas.

MATERIALS AND METHODS

Case series and histology

Consecutive formalin-fixed paraffin-embedded head and neck lesions diagnosed as squamous cell papillomas between April 2010 and October 2015 were retrieved from the archives of the Pathology Department of the Regina Elena National Cancer Institute. Hematoxylin-eosin stained sections were reviewed by 2 pathologists. They confirmed that, based on the criteria of the WHO, the retrieved cases could not be classified differently from squamous cell papillomas. The cases with enough residual material for HPV testing were included in the study. Patients had provided a general consent for research use of surplus tissue at the time of biopsy collection. The study was approved by the Ethics Committee of the Regina Elena National Cancer Institute (CE/485/12).

Human papillomavirus testing

Two to 3 sections of 5 μm were obtained from each formalin-fixed paraffin-embedded block. Total nucleic acid extraction was performed using the DNeasy Blood and Tissue Kit (Qiagen, Milan, Italy), as previously described.[12] Detection and genotyping of mucosal HPVs were performed using the Inno-LiPA HPV Genotyping Extra kit (Fujirebio Italia srl, Pomezia, Italy), which detects 28 different genotypes. This assay includes a human DNA control (*HLD-DPB1* gene) to monitor sample quality and extraction efficiency. A total of 10 μl of extract was used as a template for amplification, in accord with the manufacturer's instructions. Hybridization steps to color development were carried out in a Profiblot T48 instrument (Tecan, Männedorf, Switzerland). HPV carcinogenic risk was classified in accordance with IARC indications for cervical cancer.[7] Specifically, the 12 HPVs of group I were considered as high-risk types: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

DNA extracts were also tested for cutaneous HPVs using type-specific multiplex genotyping assays (IARC, Lyon, France), which combine a multiplex polymerase chain reaction (PCR)[13] with a bead-based Luminex technology.[14,15] The presence of 79 cutaneous HPVs included in the alpha, beta, gamma, and mu genera was investigated. Amplification of β-globin was used as a positive control for the quality of template DNA.

Statistical analyses

A Fisher exact test was used to compare proportions; p values $< .05$ were considered as statistically significant. The SPSS+ statistical package version 19.0 was used for the analyses.

RESULTS

Study group

Eighty-three squamous cell papillomas were retrieved for this study. The median age of the patients was 40 years (interquartile range, 36–51 years) and more than half of the cases (53/83; 63.8%) occurred in men. Overall, 43 cases (51.8%) involving the oropharynx and 31 cases (37.4%) of the oral cavity were included (Table 1). Only a minority of cases were localized at the larynx and nasopharynx. The most frequent localization was the oral tongue.

Mucosal human papillomavirus prevalence

All the 83 papillomas were analyzed to assess the presence of mucosal HPV types and 24 cases (28.9%) tested positive. All these lesions had a single infection (Table 2). HPV prevalence was significantly higher among men than women (39.6% vs 10.0%; $p = .005$; data not shown). Only 3 of 43 cases (6.9%) of oropharyngeal papillomas harbored mucosal HPV DNA (1 tonsillar papilloma, 1 of the soft palate, and 1 of the uvula), whereas the infection was detected in 15 of 31 cases (48.3%) of oral lesions, with a significant

TABLE 1. Distribution of the 83 head and neck squamous cell papillomas by anatomic and specific subsite.

Anatomic site	No. of cases (%)	Specific subsite, *n* (%)
Oropharynx	43 (51.8)	tonsils, tonsillar pillars, tonsillar fossae, 14 (32.6)
		uvula, 16 (37.3)
		soft palate, 10 (23.2)
		posterior wall, 1 (2.3)
		base of tongue, 2 (4.6)
Oral cavity	31 (37.4)	oral tongue, 18 (58.1)
		hard palate, 5 (16.1)
		cheek mucosa, 3 (9.7)
		labial mucosa, 5 (16.1)
Larynx	6 (7.2)	
Nasopharynx	3 (3.6)	

difference in the prevalence between these 2 anatomic sites ($p < .001$). All but one of the HPV-positive oral papillomas occurred at the labial mucosa or mobile tongue. Four cases of laryngeal (4/6; 66.7%) and 2 cases of nasopharyngeal papilloma (2/3; 66.7%) were also HPV-positive.

Both low-risk and high-risk types were found in our case series. The low-risk HPV6 was the most common genotype, being present in 15 of the 24 HPV-positive lesions (62.5%). The high-risk type HPV16 was detected in 3 oral papillomas, whereas the undetermined risk type HPV74 was detected in 2 lesions. All the other types (HPV11, 18, 35, and 51) were evidenced in 1 case each.

Cutaneous human papillomavirus prevalence

Apart from 2 oropharyngeal lesions, for which residual material was not available, all the other papillomas ($n = 81$) were also tested for the presence of cutaneous HPV types. These were found in 16 of 81 cases (19.7%; Table 3). Their prevalence was higher in oral than oropharyngeal papillomas, although not significantly (22.6% vs 14.7%; $p = .5$). Only HPVs of the beta and gamma genera were detected, with a predominance of the beta types (13.6% vs 7.4%). No significant difference in cutaneous HPV prevalence was observed when comparing men and women (25.5% vs 10.0%; $p = .15$; data not shown).

The genotype-specific prevalence for the cutaneous HPVs is also shown in Table 3. Beta HPV12 and 23 were the most frequent types (3 cases each; 3.6%). All the other beta and gamma HPVs were detected in 1 or 2 lesions each.

Considering the 81 papillomas that were tested for both cutaneous and mucosal HPV types, 35 cases (43.2%) were HPV-positive: 11 lesions (13.5%) only harbored cutaneous types; 19 (23.5%) only mucosal types; and 5 (6.2%) were positive for both cutaneous and mucosal HPVs. Multiple (>1 cutaneous HPV) and mixed (cutaneous and mucosal HPVs) infections were found in 7 lesions: 1 oral and 1 oropharyngeal papillomas harbored 2 cutaneous HPVs, whereas the remaining 5 lesions (4 oral and 1 laryngeal) were positive for 1 mucosal HPV together with at least 1 cutaneous type.

DISCUSSION

HPV testing is not routinely performed on head and neck papillomas, probably because of their predominantly benign nature. This means that the exact contribution of HPV to their development remains a controversial issue. In order to clarify to which extent HPV plays a role in the occurrence of these lesions, we analyzed a moderately sized collection of lesions with a histological diagnosis of squamous papillomas. To our knowledge, this is the first study that investigates the presence of 107 different HPV types: we analyzed 83 cases for the presence of 28 mucosal HPVs, and 81 of these lesions were also tested for the presence of 79 cutaneous HPVs. Numerous other studies have investigated HPV infection in head and neck papillomas, but none of them has assessed the presence of so many different types and most of them analyzed <50 lesions.[16–20] A large variety of HPV detection methods were used (eg, immunohistochemistry, in situ hybridization, and PCR), so prevalence ranged widely from 0% to 100%.[4,17–22] Such a large variation in the HPV detection rate may also be due to the incorrect categorization of all

TABLE 2. Mucosal human papillomavirus infection, overall, by carcinogenic risk and genotype, in the entire case series of 83 squamous cell papillomas, stratified by anatomic site.

	Mucosal HPV infection No. of positive papillomas (%)				
HPV genotype	Oropharynx $n = 43$	Oral cavity $n = 31$	Larynx $n = 6$	Nasopharynx $n = 3$	All sites $n = 83$
Low-risk	1 (2.3)	9 (29.0)	4 (66.7)	2 (66.7)	16 (19.3)
6	1 (2.3)	9 (29.0)	4 (66.7)	1 (33.3)	15 (18.1)
11	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	1 (1.2)
High-risk	1 (2.3)	5 (16.1)	0 (0.0)	0 (0.0)	6 (7.2)
16	0 (0.0)	3 (9.7)	0 (0.0)	0 (0.0)	3 (3.6)
18	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (1.2)
35	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (1.2)
51	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Undetermined	1 (2.3)	1 (3.2)	0 (0.0)	0 (0.0)	2 (2.4)
74	1 (2.3)	1 (3.2)	0 (0.0)	0 (0.0)	2 (2.4)
Overall	3 (6.9)	15 (48.3)	4 (66.7)	2 (66.7)	24 (28.9)

Abbreviation: HPV, human papillomavirus.

TABLE 3. Cutaneous human papillomavirus infection, overall, by genus and genotype in 81 squamous cell papillomas, stratified by anatomic site.

	Cutaneous HPV infection No. of positive papillomas (%)				
HPV genus and genotype	Oropharynx $n = 41$	Oral cavity $n = 31$	Nasopharynx $n = 3$	Larynx $n = 6$	All sites $n = 81$
Alpha	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Beta	4 (9.8)	5 (16.1)	1 (33.3)	1 (16.6)	11 (13.6)
5	0 (0.0)	1 (3.2)*	0 (0.0)	0 (0.0)	1 (1.2)
12	2 (4.9)	1 (3.2)*	0 (0.0)	0 (0.0)	3 (3.6)
23	1 (2.4)	2 (6.4)	0 (0.0)	0 (0.0)	3 (3.6)
93	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	1 (1.2)
96	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (1.2)
98	0 (0.0)	0 (0.0)	0 (0.0)	1 (16.6)	1 (1.2)
110	0 (0.0)	1 (3.2)†	0 (0.0)	0 (0.0)	1 (1.2)
120	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Gamma	2 (4.9)	3 (9.7)	0 (0.0)	1 (16.6)	6 (7.4)
121	0 (0.0)	1 (3.2)†	0 (0.0)	0 (0.0)	1 (1.2)
123	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (1.2)
130	0 (0.0)	1 (3.2)‡	0 (0.0)	1 (16.6)	2 (2.4)
131	1 (2.4)§	1 (3.2)‡	0 (0.0)	0 (0.0)	2 (2.4)
156	1 (2.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
SD2	1 (2.4)§	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.2)
Mu	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Overall	6 (14.7)	7 (22.6)‖	1 (33.3)	2 (33.3)	16 (19.7)‖

Abbreviation: HPV, human papillomavirus.
* One case coinfected by HPV5 and 12.
† One case coinfected by HPV110 and 121.
‡ One case coinfected by HPV130 and 131.
§ One case coinfected by HPV131 and SD2.
‖ These sums are due to the abovementioned cases of coinfections.

papillary lesions as "papillomas." As already underlined, the differential diagnosis may be challenging, given that the differences in the histopathological features may be subtle, despite the diagnostic criteria indicated by the WHO.[1]

We evidenced that around one third of the cases (28.9%) harbored mucosal HPV types. Notably, these types were found in 48.3% of the oral lesions, a significantly higher prevalence compared with the oropharyngeal ones (6.9%). This finding suggests that HPV infection causes benign lesions more frequently in the oral cavity than in the oropharynx. A possible explanation is that the mucosal epithelium of the oral cavity is more accessible to HPV than the oropharyngeal one. Nevertheless, in a previous study conducted by our group on cytobrushings collected from healthy individuals, none of the oral cytobrushings tested HPV-positive, whereas HPV infection was found in 8.8% of the oropharyngeal samples.[23] Indeed, oral HPV infection is quite rare in the general population. A systematic review reported that oral HPV DNA was only present in 4.5% of 4070 healthy subjects.[24] However, because HPV infection is often searched in oral rinses/gargles, it is not possible to know the exact anatomic site of this infection (oral vs oropharyngeal).

In our cases series, the most frequent type was low-risk HPV6 (18.1%), which represents the most prevalent HPV in laryngeal lesions,[20] and is also one of the most frequent types in oral papillomas.[16,21] HPV6 usually infects the anogenital area and plays a major role, together with HPV11, in the development of anogenital warts. This suggests that HPV6-positive lesions may have resulted from an infection acquired through oral sexual contact.

Although low-risk types were found more frequently, 7.2% of the lesions analyzed harbored high-risk HPVs. HPV16, in particular, was detected in 3 oral papillomas. Other studies have already reported the presence of this genotype in benign head and neck lesions.[18,19,25] Notably, HPV16, which is rarely present in the oral cavity of healthy individuals,[23,24] represents the genotype most commonly associated with oropharyngeal cancer.[26,27]

Cutaneous HPVs were found in 19.7% of the cases analyzed. Differently from what we observed for mucosal types, their prevalence in oral and oropharyngeal lesions was similar. Therefore, in oropharyngeal lesions, cutaneous HPVs prevailed over mucosal HPVs, whereas mucosal types were predominant in oral papillomas. The presence of cutaneous HPVs in head and neck lesions is not surprising. In fact, HPV2, 4, and 10 are notably associated with oral verruca vulgaris.[1] In our samples, 14 different cutaneous HPVs were detected. Although some of them have already been reported in head and neck lesions (eg, HPV98), other genotypes, which have been isolated and characterized only recently, have only been found in oral rinses of healthy individuals or in skin samples (eg, HPV120). Importantly, our findings confirm that HPVs classically recognized as cutaneous types may be present at mucosal sites, suggesting for these genotypes a broader spectrum of target tissues than previously thought.[28] Only cutaneous genotypes of the beta and gamma genera were found in this study, and beta types were more common

both in the oral and oropharyngeal lesions. To date, no clear data are available on the possible pathogenic role of cutaneous HPVs in the development of benign or malignant head and neck lesions, even though these types have been detected in head and neck SCC[29] and esophageal carcinoma.[30] In our study, the most common cutaneous types were beta HPVs 12 and 23, which, together with HPV5, are indicated as epidermodysplasia verruciformis-associated HPV, because they can be found in nonmelanoma skin cancer of individuals affected by this rare genetic disorder.[31]

Based on our results, we can hypothesize that around one third of the lesions diagnosed as squamous papillomas represented oral condylomas, because they harbored mucosal HPVs. Given their risk of recurrence, it might be useful to identify these lesions precisely in order to distinguish them from other nonrecurrent lesions. To this aim, HPV testing may represent a useful tool. However, its use may not be indicated in the routine diagnostic process of all head and neck lesions, but should be limited to those cases with clinical and histological features insufficiently distinctive for classification purposes. Even if HPV testing may aid in the differential diagnosis of head and neck lesions, it must be noted that the most appropriate method to recognize their possible viral origin should be investigated. Even in the case of oropharyngeal SCC, for which assessment of HPV tumor status is assuming an increasing importance because of the prognostic value of HPV infection in this neoplasia, the best HPV detection method has not been established yet.[32,33]

In the present study, the samples were only tested for viral DNA. However, its presence may not be sufficient to establish a viral etiology for the HPV-positive papillomas. On the other hand, detection of HPV transcripts, which indicate an active infection, may support the etiologic role for the HPV infection and may help exclude the presence of HPV DNA as a mere contamination or as an inactive infection with no causal role in the development of the lesion.

In conclusion, the present study, using highly sensitive methods for the detection of HPV DNA in squamous cell papillomas of the head and neck region, made it possible to reveal a viral origin for almost half of these lesions considering both cutaneous and mucosal HPV infection. Additionally, it evidenced the contribution from cutaneous genotypes, commonly neglected when evaluating the mucosal surfaces. Nonetheless, viral etiology cannot be taken into consideration for a large fraction of these lesions, which remain of unknown origin.

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