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HPV in Oropharyngeal Squamous Papillomas: A Missing Link in Head and Neck Viral Pathogenesis

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Human papillomavirus (HPVs), oropharyngeal papilloma, oropharyngeal carcinoma, viral oncogenesis, E4/p16 protein expression.

ABSTRACT

The role of human papillomaviruses (HPV) in oropharyngeal squamous papillomas remains incompletely defined, in contrast to their established involvement in oropharyngeal squamous cell carcinoma and laryngeal papillomatosis. This study aimed to assess HPV prevalence, genotype distribution, and associated biomarker expression in oropharyngeal papillomas, with particular emphasis on p16 and viral E4 protein immunoreexpression.

We retrospectively analyzed 51 formalin-fixed paraffin-embedded oropharyngeal squamous papillomas diagnosed between 2015 and 2023. HPV DNA detection and genotyping were performed using the INNO-LiPA assay. Immunohistochemistry for p16 and E4 was correlated with HPV status. For comparison, 20 oropharyngeal squamous cell carcinomas were included as controls, comprising 10 HPV16-positive and 10 HPV-negative cases.

HPV DNA was detected in 57% of papillomas, predominantly high-risk genotypes, most frequently HPV16. E4 immunoreexpression was observed in 55.5% of evaluable cases and was significantly associated with HPV DNA positivity ($p < 0.0001$). In contrast diffuse p16 overexpression was uncommon and showed no significant association with HPV detection. The prevailing immunophenotype in HPV-positive papillomas was E4+/p16-, whereas HPV16-positive carcinomas uniformly exhibited a p16+/E4- profile.

No statistically significant differences in recurrence, dysplasia, or malignant transformation were observed between HPV-positive and HPV-negative papillomas, although adverse events occurred exclusively in high-risk HPV-associated cases.

These findings suggest that a substantial subset of oropharyngeal squamous papillomas is associated with productive high-risk HPV infection, biologically distinct from transforming infections seen in carcinoma. While combined E4/p16 immunoprofiling may contribute to lesion characterization and prognostic stratification, its clinical utility warrants validation in larger, prospective cohorts.

INTRODUCTION

Human papillomaviruses (HPV) are a diverse group of epitheliotropic DNA viruses that infect the skin and mucosal surfaces. Some twenty HPVs in the AlphaPV genus are classified as oncogenic for humans and are often referred to as high-risk HPV (HR-HPV). Oncogenic HPV are evolutionary related and include HPV16 and HPV18 and their close relatives[1,2]. Chronic infections with HR-HPV, chiefly HPV16, are well established as causal agents in a subset of oropharyngeal squamous cell carcinomas (OPSCC)[3,4]. Another clade within AlphaPV includes HPV typically associated with benign proliferative epithelial lesions, including squamous papillomas[5]. In particular, recurrent respiratory papillomatosis (RRP) involving the larynx has been strongly linked to such non-oncogenic HPVs[6,7] often referred to as low-risk HPV (LR-HPV).

Viral proteins are differentially translated throughout the natural history of the HPV infection. In oncogenic HPV, translation of the E7 viral oncoprotein is strongly associated with overexpression of the human p16 protein, which serves as a surrogate marker for oncogenic HPV activity and has both diagnostic and prognostic relevance in HPV-induced OPSCC[8,9].

Expression of the viral E4 protein depends on keratinocytes differentiation, and is detected at high levels in the epithelial squamous layer[10], being often used as a marker of active viral gene expression[11,12]. Progression to high grade and cancerous lesions concurs with differentiation loss in the transformed cells, and thus with a loss of E4 expression. The E4 viral protein is thus regarded as a general marker for the onset of the viral replicative stage as well as a marker for low grade lesions[13,14].

Of interest, combined p16/E4 immunohistochemistry can stratify HR-HPVs lesions according to the progression of the natural history of the infection. Indeed, the combined p16 and E4 expression patterns have been investigated in anal intraepithelial neoplasia (AIN) and in cervical intraepithelial neoplasia (CIN). Characteristically, AIN1 and CIN1 lesions show extensive E4 staining and patchy p16 positivity in the lower one third of the epithelium whereas AIN3 and CIN3 lesions show no to focal E4 staining and display full-thickness p16 positivity[15–17]

Despite the extensive characterization of the aetiological role of oncogenic HPV in both malignant and benign lesions of the head and neck, the role of HPV in oropharyngeal squamous papillomas remains poorly defined[18]. Most existing studies have focused either on malignant lesions of the oropharynx or on benign lesions of the larynx, with a relative paucity of data on benign oropharyngeal tumors. Consequently, the prevalence of HPV infection, the viral genotype spectrum, as well as the viral protein expression profiles in oropharyngeal squamous papillomas remain largely unexplored.

Understanding the involvement of HPV in these lesions is important not only for clarifying their etiology but also for exploring potential diagnostic biomarkers. In particular, examining HPV DNA positivity together with the expression profiles of infection-related proteins such as viral E4 and human p16 could provide insights into viral activity and pathogenic mechanisms.

The aim of this study is to investigate the prevalence of HPVs in squamous papillomas of the oropharynx, to characterize the distribution of HPVs genotypes, and to analyze E4 and p16 immunexpression. Additionally, we seek to explore the correlation between viral DNA presence and the expression of these biomarkers. We also seek to identify potential prognostic differences between HPV-positive and HPV-negative forms, thereby contributing to the understanding of HPV's potential role in the pathogenesis of these benign oropharyngeal lesions.

MATERIAL AND METHOD

Case selection

We retrospectively collected biopsy and/or surgically resected specimens of formalin-fixed paraffin-embedded (FFPE) tissues from patients with oropharyngeal squamous papillomas (hereafter referred to as papilloma) from the Department of Pathology, Centre Hospital Universitaire (CHU), Montpellier, France, between 2015 and 2023.

All available slides were centrally reviewed, and only cases fulfilling strict histopathological criteria for squamous papilloma were included. These criteria comprised an exophytic papillary architecture with well-formed fibrovascular cores lined by mature stratified squamous epithelium. Cases consisting exclusively of superficial squamous fragments without identifiable fibrovascular cores, lesions showing predominantly non-specific squamous hyperplasia, or cases considered histologically equivocal were excluded from the study

The clinical information of the patients was obtained from their medical records. This study was approved by the Institutional Review Board of CHU Montpellier (No A086/2025-04-247/001)

As a control for immunochemistry analyses we included 20 cases of OPSCC (10 HPV16+ and 10 HR-HPVs-) and compared E4 and p16 protein expression with those of papillomas.

Histological evaluation

Four pathologists (V.L.S., V.C.M., A.T., and C.J.) including two board-certified pathologists with expertise in head and neck pathology (V.L.S and V.C.M) reviewed and made the diagnoses

according to the World Health Organization (WHO) classification of Head and Neck Tumors[19].

In cases with limited or fragmented sampling, particular attention was paid to the presence of fibrovascular cores to confirm the diagnosis of papilloma and to exclude other exophytic or hyperplastic squamous lesions. The differential diagnosis with condyloma-like lesions was based on architectural features (papillary fronds with central fibrovascular cores versus broad-based verruciform growth) and epithelial maturation pattern. When clinicopathological correlation was insufficient and morphology was equivocal, cases were excluded.

The presence of koilocytes, as well as the presence and degree of dysplasia were evaluated. Dysplasia was assessed within the papillary epithelium of the lesion and required unequivocal architectural and cytological atypia, including nuclear enlargement, hyperchromasia, loss of polarity, and increased mitotic activity, exceeding the spectrum of reactive or HPV-related changes. When present, dysplasia was classified using a two-tiered system as low-grade or high-grade.

p16 and E4 immunohistochemistry

Immunohistochemistry was performed using 4- μ m thick FFPE tissue sections and primary monoclonal antibodies against p16 (E6H4, prediluted, CIN Histology Kit; Roche, Heidelberg, Germany) and E4 (SILgrade-E4 (XR-E4-1), prediluted, DDL Diagnostic Laboratory B.V., Rijswijk, Nederland) according to the manufacturers' protocols.

The SIL grade-E4 is a pan-genotypic monoclonal antibody reactive against the HPV E4 protein from at least HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV42, HPV43, HPV44, HPV45, HPV51, HPV52, HPV53, HPV56, HPV58, HPV59, HPV66, HPV67, HPV70 and HPV74; and skin HPV genotypes 27 and 57. E4 protein immunoexpression was scored as previously reported in cervical and anal lesions[14,15], briefly: negative (score 0); focal: restricted to groups of cells in the upper layers of the epithelium (score 1); and extensive: upper half of the epithelium or more (score 2). Because E4 is a late viral protein expressed during replicative infection and physiologically restricted to differentiated epithelial layer, even focal staining in the superficial epithelium was considered biologically positive.

P16 was considered positive if there was at least 70% nuclear and cytoplasmic expression with strong intensity as recommended[20].

FFPE sample preparation and nucleic acid extraction

FFPE tissue samples were sectioned for histological and DNA analyses. For DNA extraction, 10 consecutive 10- μ m sections were transferred using a sterile wooden stick into two 1.5-mL

Eppendorf tubes. FFPE blocks were processed under strict pre-/post-PCR conditions (physical separation), and blank paraffin blocks were systematically tested in parallel to serve as sentinels for contamination.

DNA was extracted using the Maxwell® RSC DNA FFPE kit (Promega, Madison, WI, USA), according to the manufacturer's recommendation. Extracted DNA was quantified using the Qubit dsDNA high sensitivity assay kit and a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and amplification of the gDNA extracted from the FFPE samples was determined by quantitative PCR (qPCR) using the KAPA SYBR FAST Master Mix Universal (Kapa Biosystems, Wilmington, USA), as previously described[21].

HPV-DNA detection and genotyping

HPV genotyping was performed in duplicate using 10 µL of DNA and an INNO-LiPA HPV Genotyping *Extra II* assay kit (Fujirebio, Les Ulis, France). This test, based on reverse hybridization after a PCR step, allows the type-specific detection of 32 viral genotypes within the *Alphapapillomavirus* genus, including oncogenic HPVs (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59), possibly oncogenic HPV (HPV68), probably oncogenic HPVs (HPV26, 53, 66, 67, 70, 73, and 82), and non-oncogenic/unclassified HPVs (HPV6, 11, 40, 42, 43, 44, 54, 61, 62, 81, 83, and 89).

Statistical analysis

Statistical tests were performed using GraphPad Prism 10.0 (GraphPad). Fisher's exact test was used to evaluate associations between different markers. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Clinicopathological characteristics of the cohort

After central histopathological review, 51 cases fulfilled the predefined diagnostic criteria for oropharyngeal squamous papilloma and were included in this study.

The clinicopathologic characteristics of the 51 cases are summarized in Table 1.

Patients ranged in age from 10 to 85 years (mean: 41.5 years), with a male to female ratio of 0.82:1. The lesions were located as followed: 50% (25/51) tonsil, 39% (20/51) uvula, 6% (3/51) base of tongue, 6% (3/51) oropharynx not otherwise specified. Histologically, none of the papillomas displayed koilocytosis. Dysplasia was evaluated in 50 cases and was present in 8% (4/50) lesions: three cases of low-grade dysplasia and one case with high-grade dysplasia. Two patients presented bilateral papillomas. One patient experienced local recurrence. One patient suffered from papilloma transformation into a squamous cell carcinoma. No patient died of disease. Follow-up data were available for all 51 patients. The median follow-up duration was 62 months (interquartile range: 23-96 months; range: 7-125 months).

HPV DNA detection and genotyping

HPV DNA was detected in 57% (29/51) of papillomas. The most prevalent genotypes were HPV16 and HPV6, detected in 51.7% (15/29) and 20.7% (6/29) of HPV-positives cases, respectively. A significant fraction of the positive samples of sample (31%, 9/29) harbored DNA from multiple HPV types.

Within HPV-positive cases, the prevalence of exclusively oncogenic HPV was 70% (20/29), compared with 20.7% (6/29) of exclusively non-oncogenic HPV, while 10.3% (3/29) of the HPV-positive samples harbored DNA from both oncogenic and non-oncogenic HPV.

Table 2 depicts HPV genotype distribution in the 29 oropharyngeal HPV-positives papillomas

Expression of p16 and E4 proteins in oropharyngeal papillomas

P16 immunoexpression was evaluated in 51 cases whereas E4 immunoexpression was evaluable in 45 cases only. Immunohistochemical analysis showed that p16 was positive in 1.9% (1/51) of cases. Patchy p16 immunoexpression involving 10-30% of epithelial cells and not meeting the predefined criteria for positivity was observed in 14/51 cases (27.5%). The E4 protein was detected in 55.5% (25/45) of papillomas: 13.3% (6/45) showed extensive E4 staining (score 2), while 42.2% (19/45) exhibited focal expression (score 1). E4 immunoexpression was observed in all evaluable non-oncogenic HPV cases (4/4, 100%) and in 18 of 21 evaluable oncogenic HPV cases (85.7%), with no statistically significant difference between the two groups (Fisher's exact test, $p=1.00$). The remaining cases were negative for E4 (score 0). Representative staining patterns are illustrated in Figure 1.

Correlation between HPV status and protein expression

The expression of E4 was significantly more frequent in HPV-positive papillomas than in HPV-negative ones (84.6% versus 15.8%; $p < 0.0001$, Fisher's exact test). When stratified according to HPV oncogenic risk category, E4 immunoexpression remained significantly associated with HR-HPV compared with non-HR-HPV cases (85.7% versus 29.2%; $p=0.00023$).

In contrast, no significant association was observed between overall HPV detection and diffuse p16 immunoexpression (3.3% versus 0%; $p=1.000$, Fisher's exact test). Diffuse p16 immunoexpression was observed exclusively in HR-HPV-positive papillomas, although this association did not reach statistical significance due to the very low number of positive cases.

Table 3 summarizes the association between HPV status (overall and according to oncogenic risk category) and individual E4 and p16 immunoexpression.

Analysis of combined E4/p16 immunoprofiles showed that no papilloma displayed E4+/p16+. The E4+/p16- profile was significantly more frequent in HPV-positive papillomas (73.3%) than in HPV-negative cases (15.8%) ($p=0.00011$, Fisher's exact test). Conversely, the E4-/p16- profile was predominantly observed in HPV-negative papillomas (84.2% versus 10%, $p < 0.0001$). The E4-/p16+ profile was rare and not significantly associated with HPV status ($p=1.000$).

When analyzed according to HPV oncogenic risk category, the distribution of combined E4/p16 immunoprofiles also differed significantly between HR-HPV and non-HR-HPV cases, primarily driven by a predominance of the E4+/p16- profile in HR-HPV papillomas ($p=0.0023$), (Table 4).

Comparison of E4 and p16 immunoexpression in oropharyngeal carcinomas HPV-positive and HPV-negative

In the control group of OPSCC ($n=20$), all HPV16-positive cases ($n=10$) showed strong and diffuse p16 staining, while none expressed E4 in the infiltrative component (Figure 2). Of interest these lesions showed focal E4 staining but no p16 positive expression in the normal epithelium at the edge of the malignant lesion. HPV-negative carcinomas ($n=10$) were negative for both p16 and E4 (Figure 2). These findings support the known transforming versus productive expression profiles of HPV-associated cancers versus benign lesions.

Prognostic comparison between HPV-positive and HPV-negative papillomas

Table 5 summarizes the distribution of adverse clinicopathological features according to overall HPV status and HPV oncogenic risk category.

Bilateral lesions were observed in 6.9% (2/29) of HPV-positive cases and in none of the HPV-negative cases ($p=0.500$, Fisher's exact test). Local recurrence occurred in 3.4% (1/29) of HPV-positive papillomas and in no HPV-negative cases ($p=1.000$). Dysplasia was identified in 6.9% (2/29) of HPV-positive and 9.1% (2/22) of HPV-negative papillomas ($p=1.000$). Malignant transformation was observed in 3.4% (1/29) of HPV-positive cases and in none of the HPV-negative cases ($p=1.000$). None of these individual comparisons reached statistical significance.

When stratified according to HPV oncogenic risk category, bilateral lesions were observed in 8.7% (2/23) of HR-HPV cases and in none of the non-HR-HPV cases ($p=0.198$). Local recurrence and malignant transformation each occurred in one HR-HPV-positive case (4.3%) and in no non-HR-HPV cases ($p=0.451$ for both comparisons). Dysplasia was observed in 8.7% (2/23) of HR-HPV papillomas and 7.1% (2/28) of non-HR-HPV cases ($p=1.000$). Again, no statistically significant associations were identified.

All adverse events occurring in HPV-positive papillomas were restricted to HR-HPV-positive cases. One patient had bilateral lesion with dysplasia and malignant transformation (HPV16). Another patient had bilateral papillomas (HPV16). The patient with local recurrence harbored papilloma with HPV16 and HPV33 DNA.

To increase statistical power, we performed an exploratory composite analysis (any adverse event: bilaterality, recurrence, dysplasia or malignant transformation). Adverse events occurred in 4/29 HPV-positive versus 2/22 HPV-negative papillomas ($p=0.688$). When restricted to HR-HPV-positive cases, adverse events were observed in 4/23 (17.4%) HR-HPV versus 2/28 (7.1%) non-HR-HPV cases ($p=0.390$).

DISCUSSION

In the present series, we report a substantial prevalence of HPV DNA (57%) in oropharyngeal papillomas, with a majority, approximately 60%, harboring DNA from oncogenic HPV genotype, notably HPV16. This contrasts markedly with laryngeal papillomatosis, which is almost exclusively associated with non-oncogenic HPV6 and HPV11 (low risk types) representing 90% of RRP[22]. This divergence likely reflects a combination of site-specific epithelial tropism, immune microenvironment, viral entry biology and exposure/transmission route. The lymphoid-rich crypt epithelium of the tonsils provides an accessible niche for HPV-16 persistence, unlike the smooth laryngeal mucosa[23]. Moreover the immune microenvironment of the oropharynx may permit immune evasion and long-term HPV16 carriage[24,25]. Genotype-specific entry receptor use and early gene regulation (E6/E7) may favor HPV16 in oropharyngeal basal cells, while HPV6/HPV11 remain episomal in larynx[26]. Furthermore, transmission patterns are different: adult sexual behavior is more strongly associated with oral HPV16 acquisition, while juvenile RRP often reflects vertical oral-genital HPV6/11 transmission[27]. Taken together, anatomical microstructure, local immunity, entry biology, and exposure patterns provide plausible explanations for why HR-HPV dominate in oropharyngeal papillomas, while LR-HPV are the hallmark of laryngeal papillomatosis.

Several previous studies have investigated HPV detection in head and neck squamous papillomas; however, data specifically focusing on oropharynx remain limited and heterogeneous. Reported HPV detection rates vary considerably across series, likely reflecting differences in anatomical site definitions, patient selection, and HPV detection techniques. Some studies have reported low HPV prevalence in oropharyngeal papillomas, whereas others have identified HPV in a subset of cases, predominantly non-oncogenic types[28–30]. Among studies addressing clinical outcomes in these lesions, occasional local recurrence has been reported whereas malignant transformation appears to be absent. Importantly, these studies primarily relied on HPV DNA detection and did not systematically integrate viral genotyping with biomarkers distinguishing replicative from transforming infection.

In this context, the combined evaluation of E4 and p16 immunorexpression in oropharyngeal papillomas has not, to our knowledge, been systematically investigated. The relative scarcity of biologically focused studies in this anatomical site may reflect the generally benign clinical course and low malignant potential of these lesions, which have historically attracted less molecular attention than HPV-associated carcinomas.

We observed a strong correlation between E4 immunorexpression and HPV DNA positivity: 84.6% of HPV-positive papillomas expressed E4, versus only 15.8% in HPV-negative cases ($p < 0.0001$). No significant difference in E4 immunorexpression was observed between oncogenic and non-oncogenic HPV types ($p = 1.00$), supporting its role as a marker of replicative infection rather than viral oncogenic risk[14]. Within oropharyngeal papillomas, E4 immunorexpression could therefore serve as an accurate surrogate for active HPV presence and replicative infection.

Interestingly, three papillomas in our cohort exhibited E4 positivity despite being HPV DNA–negative by INNO-LiPA genotyping. Several explanations may account for these discordant cases. First, false-negative HPV DNA results may arise from low viral load or DNA degradation in FFPE tissues. In addition, the INNO-LiPA assay targets only a subset of α -HPV genotypes and does not include certain cutaneous types such as HPV27 and HPV57. In contrast, the pan-E4 antibody used in this study (SILgrade-E4) has been validated to cross-react with E4 proteins from multiple genotypes, including HPV27 and HPV57. Finally, abortive infections or focal viral genome loss have been described, in which E4 protein expression persists in differentiated cells despite absence of detectable viral DNA[10].

In HPV-positive oropharyngeal papillomas, the dominant profile was E4+/p16-. Patchy p16 immunoexpression was observed in a subset of papillomas but remained below the threshold required for diffuse overexpression and positivity, supporting a predominantly non-transforming infection pattern in these lesions. In contrast, HPV16+ oropharyngeal carcinomas exhibited p16 positivity without E4 expression. This suggests that papillomas frequently exhibit a replicative, non-transforming stage in the natural history of the infection, whereas carcinomas demonstrate a transforming phenotype. This dichotomy parallels with observations in AIN and CIN, where low-grade lesions express E4 and have patchy p16, while high-grade transforming lesions shows p16 positive immunoexpression and little to no E4[16]. The apparent discrepancy with the classical progression model described in VIN and AIN deserves comment. In intraepithelial neoplasia, replicative HPV infection may evolve toward transforming lesions within a relatively confined epithelial compartment. In contrast, squamous papillomas represent exophytic lesions characterized by orderly epithelial maturation and limited basal compartment expansion. This architectural context may restrict the clonal selection of cells harboring transforming HPV events. In addition, replicative HPV infection in papillomas is typically associated with lower viral load, preserved epithelial differentiation, and maintenance of the viral genome in episomal form, conditions that are less conducive to genomic instability and integration. Finally, local immune surveillance and lesion microenvironment may further influence whether oncogenic-HPV infection remains replicative or progresses toward transformation. Together, these factors suggest that, despite involvement of similar squamous epithelium, the biological trajectory of oncogenic-HPV infection may differ substantially between exophytic papillomas and intraepithelial neoplasia. Among the cohort, two patients deserve particular mention due to their unusual biomarker profiles and clinical courses. Patient 1 presented with a p16+/E4- papilloma (HPV16+/HPV33+). During follow-up, he experienced multiple local recurrences but did not develop malignant transformation. Careful histopathological re-evaluation of the original specimen, including deeper sections, as well as subsequent excision specimens obtained during follow-up, confirmed the absence of stromal invasion or destructive growth. The lesion retained an exophytic papillary architecture with preserved epithelial maturation, supporting the diagnosis of squamous papilloma. Although the p16+/E4- profile is generally considered indicative of a transforming-type HPV infection, the absence of carcinoma in this case suggest

that additional host or viral factors, such as effective local immune surveillance or low levels of viral oncogene transcription may modulate disease progression despite a “transforming” biomarker pattern[31]. Patient 2 had a papilloma with a p16-/E4+ profile. The lesion was positive for oncogenic HPV (HPV16). Although classified as p16-negative according to the predefined $\geq 70\%$ threshold, focal p16 immunoreexpression involving 30% of epithelial cells was observed. This patient developed squamous cell carcinoma less than one month after the initial diagnosis. Given this very short interval the possibility of a synchronous lesion or sampling limitation at the time of the initial biopsy cannot be excluded. Although the E4+/p16-profile is typically associated with replicative, non-transforming HPV infection, this case should therefore be interpreted with caution. Possible explanations include the presence of an early invasive component not represented in the initial specimen, the coexistence of an undetected integral viral clone or contributory host/microenvironmental factors[31].

From a clinical standpoint, although adverse events were numerically more frequent in oncogenic-HPV-positive papillomas, none of the individual prognostic parameters reached statistical significance. An exploratory composite analysis grouping all adverse features (bilaterality, recurrence, dysplasia or malignant transformation) showed a similar non-significant trend with wide confidence intervals. These findings most likely reflect the very low event rate and limited statistical power rather than true absence of association. Therefore, any prognostic interpretation should be made cautiously, and larger multicenter cohorts will be required to clarify potential clinical impact of oncogenic-HPV in oropharyngeal papillomas. The assessment of epithelial dysplasia in the oropharynx warrants caution. In this study, dysplasia was recorded conservatively and only in the presence of unequivocal architectural and cytological abnormalities within exophytic papillomas.

Pathophysiological mechanisms underlying E4 and p16 protein expression are well known. E4 is a viral protein expressed during the replicative HPV viral cycle associated with viral genome amplification and concurrent in time with capsid protein synthesis. It facilitates virion release by disrupting host cell intermediate filaments and optimizing viral replication efficiency[32]. In contrast, HR-HPV-mediated transformation, as seen in carcinomas, involves early viral oncoprotein E6 and E7, which disrupt tumor suppressors p53 and pRb, respectively. This leads to p16 upregulation due to feedback loss of pRb-mediated repression, culminating in the transforming phenotype[33,34].

In contrast to p16, which serve as a surrogate marker of transforming infection and requires diffuse overexpression to be considered positive, E4 immunoreexpression is inherently focal and limited to differentiated epithelial layers during replicative phase. Therefore, even limited E4 staining reflects active viral genome amplification rather than background expression.

Of interest, in the present work, the same HR-HPV, particularly HPV16, can be associated with either benign papillomas or malignant carcinomas in the oropharynx. The duality observed where the same HR-HPV (notably HPV16) can cause both benign oropharyngeal papillomas

and oropharyngeal malignant carcinomas may be rooted in the viral life cycle's endpoint: productive episomal infection versus malignant genome integration. Integration of HR-HPV DNA into the host genome is a central step toward malignancy, driving genomic instability, immune escape, and sustained E6/E7 oncogene expression[35,36]. In oropharyngeal carcinomas, viral genomes may exist in episomal, integrated, or mixed states[37]. In contrast, replicative HPV infection in benign lesions tends to maintain viral episomes without integration, favoring transient E6/E7 expression and permitting differentiation-dependent viral replication without transformation[38].

Several mechanisms may explain inter-patient differences in viral physical state. Integration is favored when host cells accumulate DNA double-strand breaks and rely on error-prone repair such as microhomology-mediated end joining (MMEJ), a process promoted by HPV16 E7 through suppression of canonical non-homologous end joining (NHEJ)[39]. Integration frequently occurs at common fragile sites and in enhancer-rich chromatin regions[40,41]. In some patients, robust E2-Brd4-chromatin tethering maintains the viral genome as an episome, supporting a productive cycle with E4 expression and preserving epithelial differentiation[42]. Integration can be selected when it confers a proliferative advantage or after therapies (radiation), that increase DNA damage and alter repair pathway use.

An additional factor that may critically contribute to the observed dichotomy between benign and malignant HR-HPV-associated lesions is the intrinsic difference between epithelial subtypes within the oropharynx. HR-HPV-positive oropharyngeal carcinomas preferentially arise from the reticulated crypt epithelium of the palatine and lingual tonsils, which differs markedly from the compact stratified squamous epithelium of the surface mucosa where papillomas typically develop. The crypt epithelium is characterized by a lacking basement membrane, dense lymphoid infiltration, and a unique microenvironment that may facilitate viral access to basal progenitor cells and alter immune surveillance. Such structural and immunological features could favor persistent infection, viral integration, and oncogenic progression. In contrast, the more organized surface epithelium may better support a differentiation-dependent productive viral cycle with episomal maintenance and E4 expression. The epithelial context may therefore represent a key determinant of whether HR-HPV infection remains replicative and benign or progresses toward transformation.

Taken together, these factors suggest that viral integration is not an inevitable outcome of HR-HPV infection, but rather the result of a convergence of host genomic instability, repair pathway choice, chromatin architecture, selective pressures and the epithelial subtype difference within the oropharynx. This model provides a plausible explanation for why, in the oropharynx, HR-HPV can give rise to either benign, E4-positive papillomas or malignant, p16-positive carcinomas.

CONCLUSION

This study demonstrates that HPV are frequently detected in oropharyngeal papillomas, with a predominance of high-risk types, particularly HPV16, contrasting with the HPV6/11 profile of laryngeal papillomatosis. E4 expression was strongly associated with HPV DNA positivity, supporting its role as a surrogate marker of replicative infection in this setting. Most papillomas displayed an E4+/p16– profile, in sharp contrast with oropharyngeal carcinomas, which were consistently p16+/E4–, underscoring the dichotomy between replicative and transforming infections.

Unusual cases were observed, including one p16+/E4– papilloma with multiple recurrences but no malignant transformation, and one p16–/E4+ papilloma that rapidly transformed into carcinoma. These findings highlight both the value and the limitations of E4/p16 profiles for predicting individual outcomes.

Overall, our results suggest that the fate of HR-HPV infection in the oropharynx—benign papilloma or carcinoma—depends on whether the viral genome persists episomally or integrates. Combined p16/E4 immunoprofiles may help guide clinical management, but prospective studies are needed to validate their prognostic utility.

COMPLIANCE WITH ETHICAL STANDARDS This study was conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study was approved by the Institutional Review Board of the Centre Hospitalier Universitaire of Montpellier (No A086/2025-04-247/001).

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CONFLICT OF INTEREST Authors declare that they have no conflict of interest

CONTRIBUTIONS VLS conceived and designed the study. VLS, VCM, AT and CJ performed histopathological review. VLS AT and CJ collected clinical and pathological data. NB performed HPV molecular analyses. VLS performed statistical analyses. VLS drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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FIGURE LEGENDS

Figure 1- A: Hematoxylin eosin staining of oropharyngeal papilloma with HPV16 (X5) showing patchy p16 expression considered as negative (B, X5) and extensive E4 protein expression, score 2 (C, X5). D: Hematoxylin eosin staining of oropharyngeal papilloma HPV negative (X10) showing neither p16 (E, X10), nor E4, score 0 (F, X10) protein expression. G: Hematoxylin eosin staining of oropharyngeal papilloma (Patient 1) with both HPV16 and HPV33 showing diffuse p16 protein expression, considered as positive (H, X10) and no E4 protein expression, score 0 (I, X10). J: Hematoxylin eosin staining of oropharyngeal papilloma with HPV6 (X10) showing no p16 protein expression (K, X10) and E4 staining in the upper layer, score 1 (L, X10).

Figure 2-A: Hematoxylin eosin staining of oropharyngeal carcinoma with HPV16 (X5) showing p16 positive expression (>70%) in the invasive carcinoma (orange arrow) whereas normal surface epithelium was negative (blue arrow) (B, X5). In this case, normal surface epithelium had extensive E4 staining (blue arrow) whereas invasive carcinoma was negative (orange arrow) (C, X5). D: Hematoxylin eosin staining of oropharyngeal carcinoma HPV DNA negative (X5). This case had no p16 protein expression (patchy staining in the normal surface epithelium considered as negative) (E, X5) and no E4 protein expression (both in the invasive carcinoma and the normal surface epithelium) (F, X5)