



THE ROLE OF HUMAN PAPILLOMA VIRUS (HPV) INFECTION IN THE DEVELOPMENT OF ORAL SQUAMOUS CELL CARCINOMA - LITERATURE REVIEW

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ABSTRACT:

Background Oral squamous cell carcinoma has an unclear etiology and most often occurs in middle-aged and elderly people. OSCC in these patients is usually associated with tobacco and alcohol consumption, local irritants, etc., while in people d" 40 years old, HPV is indicated as a risk factor for the occurrence and progression of OSCC.

Aim We aimed to investigate the relationship between HPV infection and the development of oral squamous cell carcinoma in more depth in the current literature as well as the different methods for the detection of HPV subtypes, which can be useful for treatment plan and prognosis.

Material and methods We have searched the following electronic databases: PubMed and Medline, mainly from 2000 to 2024. We included English language articles only. We hand-searched the reference lists of the studies retrieved and journals in the area of HPV, molecular mechanism of cancerogenesis and methods for detection of HPV in oral samples.

Results 71 articles were included and analyse in this review. HPV's mechanism of action, persistence in the host, clearance, and underlying molecular mechanisms are still not well understood and are under investigation. Transformative molecular pathways involving E6 and E7 genes are well known, leading to HPV-induced carcinogenesis, and another oncoprotein, E5, associates with EGFR epidermal growth factor receptor (cognate cell surface receptor) in the pathogenesis of oral carcinoma. Different detection methods are used, but their accuracy is controversial.

Conclusion Early diagnosis of HPV infection-related oral carcinoma is important to establish timely treatment plan and to improve the prognosis.

Keywords: HPV genotype, carcinogenesis, detection methods, oral squamous cell carcinoma, prevalence risk factors, prognosis,

INTRODUCTION

The prevalence of oral squamous cell carcinoma cancer (OSCC) statistics varies widely worldwide geographically because of different lifestyles and exposure to various risk factors- in Asia (65.8%), followed by Europe (17.3%), North America (7.3%), Latin America and the Caribbean (4.7%), Africa (3.8%), and Oceania (1.3%) [1] Several risk factors are associated with OSCC, including tobacco use, alcohol consumption, and infection with high-risk HPV genotypes and molecular signalling transducers. [2, 3, 4]. HR-HPV+ and HR-HPV- Head and Neck Squamous Cell Carcinomas (HNSCCs) possess distinct clinical and molecular-genetic characterization with different risk factors. HR-HPV frequency in HNSCC overall is influenced by the study population, site of the tumors, and detection methods [5, 6]. In low percentages, high-risk-HPV positivity could be an independent risk factor for HNSCC (head and neck squamous cell carcinoma), especially OSCC (oral squamous cell carcinoma) [6].

Human papillomaviruses (HPV) are epithelotropic viruses with a marked affinity for keratinocytes, primarily affecting the anogenital tract, skin, larynx, tracheo-bronchial system, and oral mucosa. The incidence of HPV in oral squamous cell carcinoma (OSCC) is variable, ranging from 2% to 5.6% [2]. While a potential link between HPV infection and malignancy has been hypothesized, definitive confirmation is insufficient.

The incidence of OSCC has risen in recent years, particularly among younger men. The prevalence of HPV infection has likely contributed to the increasing trend of HPV-positive patients with OSCC.

AIM:

The aim of our review is to investigate the relationship between HPV infection and the development of oral squamous cell carcinoma in more depth in the current literature, as well as the different methods for the detection of HPV subtypes, which can be useful for treatment plan and prognosis.

MATERIAL AND METHODS:

We have searched the following electronic databases: PubMed and Medline, mainly from 2000 to 2024. We included English language articles only. We hand-searched the reference lists of the studies retrieved and journals in the area of HPV, molecular mechanism of cancerogenesis and methods for detection of HPV in oral samples.

Selection criteria:

We included randomized controlled trials, literature reviews and studies involving HPV and head and neck squamous cell carcinoma (HNSCC)

Exclusion criteria:

Articles published in a language other than English, timeframe before 2000 year, non peer reviewed journal articles.

Data collection and analysis:

Two review authors independently extracted data from selected studies. This literature review will summarize the current up-to-date information.

RESULTS

71 articles were included and analysed in this review. HPV's mechanism of action, persistence in the host, clearance, and underlying molecular mechanisms are still not well understood and are under investigation. Transformative molecular pathways involving E6 and E7 genes are well known, leading to HPV-induced carcinogenesis, and another oncoprotein, E5, associates with EGFR epidermal growth factor receptor (cognate cell surface receptor) in the pathogenesis of oral carcinoma. Different detection methods are used, but their accuracy is controversial.

HPV is a small, non-enveloped, with a diameter of 52–55 nm. Its genome, housed within a non-enveloped protein capsid, consists of 8kb of double-stranded DNA molecule associated with cellular histones [2, 3, 4].

HPV-DNA genome encodes about eight open reading frames (ORF). Three functional regions are detected in ORF: the Early (E) Region comprising 45% of the genome, the Late (L) Region comprising 40% of the genome, and the Long Control Region (LCR).

The Early ORF encode proteins E1, E2, E4, E5, E6, and E7, which are critical for replication, cellular transformation, and regulation of viral transcription. The role of E1 and E2 are to maintain viral DNA and aid in genome segregation during cell division. Proteins E6 and E7 promote cell cycle progression during productive infection. E1, E2, E4, and E5 proteins are essential because they are expressed during the amplification of viral DNA in well-differentiated cells located in the upper layers of

the epithelium. The Late Region encodes structural (capsid) proteins required for virion assembly [5-8]. Based on pathogenic potential, HPV is divided into low-risk HPV genotype (LR- HPV) and high-risk HPV genotype (HR- HPV). (4)

Low-risk HPVs, mainly HPV-6 and HPV-11, are associated with some oral benign papillomatous lesions, including oral verruca vulgaris, oral squamous papilloma, condyloma accuminatum, and focal epithelial hyperplasia (Morbus Heck). (6-9)

High-risk HPVs, HPV-16 and HPV-18, are linked to oral potentially malignant disorders (OPMDs) and OSCC [5,6,7,8]. HPV-16 is particularly associated with oral leukoplakia (OL), including proliferative verrucous leukoplakia (PVL), a lesion with a high malignant potential. The reported rates of HPV detection in OPMD and OSCC vary widely, influenced by ethnicity, geographic location, and differences in detection methodologies [6, 7, 8, 9].

HPV-positive OSCCs exhibit distinct molecular profiles compared to HPV-negative carcinomas. Currently, no standardized procedure definitively diagnoses HPV-induced head and neck carcinomas. Even with extensive laboratory testing, determining whether a tumor is HPV-induced can be challenging in individual cases [9, 10, 11].

A wide range of methods are used to detect HPV, including Polymerase Chain Reaction (PCR), In Situ Hybridization (ISH) (allows direct detection of HPV DNA in tumor cells and has the advantage of identifying the presence and location of viral DNA in tumor cells, though it has limited sensitivity), Immunohistochemistry (IHC) and Western Blot. However, PCR has established as the most widely used method for assessing sample HPV subtypes. [7, 11, 12] Apart from the method used, results can vary depending on the use of fresh-frozen versus formalin-fixed, paraffin-embedded materials [7, 10, 12].

Recent studies have explored detecting HPV in saliva, tumor tissue and oral exfoliated cells, but the specificity and sensitivity of these methods remain questionable [5, 7, 8, 13].

HPV-positive tumors are often associated with advanced stages, including large metastatic lymph nodes [9,14].

However, studies have consistently demonstrated that HPV-positive neoplasms in the upper aerodigestive tract have a better prognosis than HPV-negative tumours. This is independent of the treatment modalities received and is more pronounced in patients who are HPV-positive and p16 positive [10, 12].

The incidence rate of HPV-positive HNSCC is steadily increasing. Accurate determination of the HPV status of tumors is critical, as HPV-positive (HPV+) and HPV-negative (HPV-) tumors are distinct biological and clinical entities that may require different treatment approaches. High-risk HPV subtypes encode the oncoproteins E6 and E7, which disrupt cellular senescence and promote tumorigenesis. Current HPV detection methods exploit this oncogenic pathway and detect the virus at different biological stages. A comprehensive re-

view of existing HPV detection techniques is necessary to better understand the role of specific high-risk HPV strains in the development of oral carcinoma. Such studies may also clarify their current or potential future contributions to patient management and prognosis [11, 12, 14, 15].

HPV-related tumors most commonly develop in the oropharynx, lymphoid tissue in the oropharynx, epipharynx, and Waldeyer's ring (e.g., the tonsils, base of the tongue, and soft palate). HPV-positive oropharyngeal cancers are clinically and biologically distinct from their HPV-negative counterparts and are associated with a more favorable overall prognosis [15, 16, 17, 18, 19]. Given this difference in prognosis and treatment implications, accurate determination of the HPV status of the tumor is of paramount importance.

High-risk HPV subtypes are well-established as tumorigenic in cervical cancer and are thought to be very similar to HNSCC, although their exact role in oropharyngeal cancer remains under investigation [18, 19].

HPV16 is the most frequently implicated subtype in head and neck tumors, with other high-risk subtypes playing lesser roles [16]. High-risk HPV subtypes can be incorporated into the human genome. Thus, the viral oncoproteins E6 and E7 are overexpressed. These proteins promote neoplasm progression by inactivating TP53 and retinoblastoma (Rb) tumor suppressor genes [21, 22, 23].

The viral oncoprotein E7 binds to the retinoblastoma protein (Rb) and disrupt the cell cycle, thus eventually initiating transcription of S-phase genes. The transition from the G1 to the S phase of the cell cycle is partially regulated by the interaction of p16 with pRb [22]. In HPV-induced carcinogenesis, pRb becomes functionally inactivated, leading to an overexpression of p16 due to the loss of negative feedback mechanisms [23].

In contrast, most head and neck squamous cell carcinomas (HNSCCs) that are not HPV-related exhibit TP53 disruptions, resulting in cell cycle dysregulation in the absence of p16 regulation [24, 25].

Studies have focused on elucidating the mechanisms by which HPV contributes to carcinogenesis. Once the viral DNA is integrated into the host cell nucleus, HPV initiates the productive phase of the viral life cycle, and this integration is important for the dysregulation of important mechanisms. The HPV genes E6 and E7 have been identified as primary oncogenic drivers in HPV-associated cancers [4, 8]. For this purpose, the expression of the viral oncoproteins E6 and E7 is important. E6 protein induces degradation of p53 through ubiquitin-mediated proteolysis, preventing apoptosis in differentiated and undifferentiated cells. E6 promotes the degradation of p53 through its interaction with E6-associated protein (E6AP). [4, 24, 25, 26] E7 binds to the retinoblastoma protein (pRb) and inhibits retinoblastoma (Rb) family members (p105 (RB), p107, and p130), releasing E2F transcription, thus causing proliferation and gene expression and disrupts its complexing with E2F, a group of genes that encode a family of transcription factors (TFs). (4, 21, 24) Both genes interact with and inhibit the activities of tumor

suppressor proteins such as p53 and pRb. One function of pRb is to prevent excessive cell growth by inhibiting cell cycle progression [24]. In HPV+ve cancers, viral E7 binds to pRb, causing its degradation and bypassing the cell cycle checkpoint. As the following mechanism, p16 expression is stimulated by E2F, resulting in its accumulation in nuclei and cytoplasm. [4, 27] The major HPV oncogenes, E6 and E7 promote uncontrolled cell growth and genomic instability by down-regulating tumour suppressor genes, p53 and retinoblastoma (Rb)

The simplest method for detecting high-risk HPV exploits this oncogenic pathway by using p16 overexpression as a surrogate marker for HPV-related malignancies [22, 26]. HPV-specific detection techniques include the detection of viral DNA via polymerase chain reaction (PCR) or in situ hybridization (ISH) or the detection of HPV RNA using reverse transcription PCR (RT-PCR) or ISH. These methods can be applied not only to tissue specimens but also to fine-needle aspiration biopsy (FNAB) specimens, saliva, and serum samples. [27, 28]

2. Determination of p16 in Tissue Specimens

Immunohistochemical (IHC) staining for p16 is a well-established and widely used surrogate marker for identifying HPV in oropharyngeal squamous cell carcinomas (OPSCCs). Recent guidelines from the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recommend testing all oropharyngeal tissue specimens for HPV status, especially high risk subtypes, with p16 testing as the initial step before proceeding to HPV-specific testing [29, 30]. Similarly, these guidelines advise routine use of p16 IHC for HPV detection in cases of squamous cell carcinomas (SCCs) with unknown primary tumours and metastatic cervical lymph nodes from the upper and middle jugular groups (levels II and III). Although there was previously no universal standard, the guidelines now define p16 positivity as >70% of tumour cells exhibiting moderately strong nuclear and cytoplasmic staining. A recent systematic review found that among patients with OPSCC, p16 IHC had a sensitivity of 94% (95% CI: 91–97%) and a specificity of 83% (95% CI: 78–88%) [30].

However, discordant cases, which account for up to 17% of OPSCCs, may reflect cancers harboring transcriptionally inactive HPV, bystander HPV from adjacent non-affected mucosa or saliva, or a completely different biological entity. Cases with discordant p16 and HPV-positive tests have been shown to have different prognostic implications. [31]

For example, two similar meta-analyses have demonstrated improved outcomes in 5-year survival without any recurrence and overall survival among patients with p16+/HPV+ OPSCCs compared to those with p16–/HPV+ or p16+/HPV– OPSCCs [32, 33, 34]. Interestingly, the prevalence of discordant cases increases when HNSCCs from non-oropharyngeal sites are tested. Consequently, p16 expression is not a reliable surrogate marker for HPV in non-oropharyngeal sites, as the prognostic value of p16 positivity in non-OPSCC cancers remains unclear [29, 30].

Despite some limitations, p16 positivity among patients with OPSCC is an important and independent prognostic biomarker [24, 32, 35, 36]. Further research is necessary to assess the prognostic significance of p16 IHC and HPV-specific testing in head and neck sites outside the oropharynx.

3. HPV ISH in Tissue Specimens

For HPV to be biologically and clinically pertinent, it must be transcriptionally active. Some researchers suggest that detecting HPV RNA should be the gold standard for HPV testing. Studies show that patients with HPV DNA+/RNA+ tumours have significantly improved overall survival compared to those with HPV DNA+/RNA- tumours [37,38]. RNA ISH can detect viral E6/E7 mRNA [35,36,38] and allows visualization of transcriptionally active virus in tumour cells. Compared to DNA ISH, RNA ISH has higher sensitivity because transcription naturally amplifies viral RNA levels, even if viral DNA is present in low copies. RNA ISH is currently the most promising HPV-specific testing method. Standardized, automated RNA ISH protocols should be implemented for clinical use in all OPSCCs, respectively OSCC. [37, 38, 39, 40, 41]

4. HPV PCR in Tissue Specimens

A variety of PCR assays are available for detecting HPV DNA in tissue specimens. Originally developed and used for cervical specimens, these assays can identify multiple HPV types, including high-risk HPV subtypes [39]. PCR is particularly effective for detecting short, fragmented DNA, which is often present after formalin fixation [40, 41].

Detection of HPV DNA by PCR has been demonstrated to have a prognostic value similar to p16 status. Patients with HPV (+) oral/oropharyngeal cancers identified through PCR exhibit improved overall and disease-specific survival compared to those with HPV(-) cancer [40, 41, 42]. However, there are two major limitations associated with using PCR for HPV DNA detection. The first one is that PCR can be overly sensitive, making it difficult to determine whether the detected HPV DNA originates from malignant tumour tissue or represents bystander virus from adjacent, non-neoplastic tissues. Several studies have reported the presence of HPV DNA in tumour-free tissue as well [41, 42, 43]. The second limitation is that PCR-based detection of HPV DNA, similar to DNA ISH, does not provide accurate information on whether the virus is transcriptionally active. To address these challenges, reverse transcription PCR (RT-PCR) has been explored to detect E6 and E7 mRNA transcripts in formalin-fixed specimens. However, this method is not yet available for routine clinical use and requires further validation through comparison with established methodologies [42, 44, 45, 46].

5. Detection of HPV DNA in Blood Samples

Over the past decade, interest in circulating tumour cells (CTCs) and cell-free circulating tumour DNA (ctDNA) has grown significantly due to the idea that a se-

rum sample or “liquid biopsy” could be used for non-invasive screening and monitoring. CTCs are released into the bloodstream from the primary tumour or metastatic sites. ctDNA typically originates from apoptotic or necrotic tumour cells, which release fragmented DNA into the blood circulation. [44, 45, 46, 47, 48]

Studies have shown that patients with HPV (+) tumours are more likely to have detectable serum HPV DNA levels using real-time PCR compared to those with HPV (-) tumours prior to treatment [43]. Interestingly, the detection of HPV DNA in serum appears to be independent of the total concentration of circulating cell-free DNA [45, 46]. A systematic review by Jensen et al. presented five different studies on plasma HPV DNA detection and reported a pooled sensitivity of 54% (95% CI: 30–75%) and a pooled specificity of 98% (95% CI: 93–99%) [45].

More recent studies have employed droplet digital PCR, a technology with sensitivity comparable to standard PCR but offering improved accuracy, and have applied it in the detection of ctDNA [46, 47]. A recent study compared plasma samples from four distinct patient populations: (1) HPV+ OPSCCs (oropharyngeal) or HPV+ anal cancer, (2) HPV- HNSCCs (head and neck), (3) HPV+ OPSCCs who had completed definitive treatment, and (4) patients without cancer [48]. All patients had pre-treatment plasma samples collected, with additional samples obtained weekly during and after definitive chemotherapy. No ctDNA was detected at baseline in the two negative control groups — patients with HPV- HNSCCs and patients without cancer, indicating 100% specificity. Among 97 patients with HPV+ OPSCCs, HPV16 ctDNA was detected in 90 individuals before treatment, yielding a sensitivity of 92%. In the subset of patients followed during treatment, HPV16 ctDNA cleared within one week for most, suggesting this approach holds potential for monitoring treatment response. Another study by Chera et al. reported similar findings, demonstrating a sensitivity of 89% and a specificity of 97% for HPV16 ctDNA [49]. The use of liquid biopsies enables molecular profiling of individual tumours, and different tumour subtypes can complement the tumour, node, metastasis (TNM) staging system [9].

6. Circulating HPV DNA as a Biomarker

While ctDNA demonstrates potential as a diagnostic tool, its role as a biomarker remains uncertain, as current evidence in the literature is inconsistent. In a subset of 99 patients with HPV+ OPSCC tumours, one study found no significant difference in 3-year survival based on pre-treatment serum HPV DNA status (as determined by real-time PCR amplification of HPV16 E6 and E7 regions) [45, 46]. Additionally, follow-up data after treatment remain inconclusive, as not all patients with HPV+ tumours exhibited detectable serum HPV DNA before locoregional recurrence. Contrary to when quantitative reverse transcription PCR is used to detect HPV16 E6/E7 expression, the presence of circulating HPV16 E6/E7 mRNA at baseline, before treatment, has been associated with worse survival outcomes [43]. Several studies sug-

gest elevated levels of HPV16 ctDNA before treatment correlate with worse prognosis [47, 53] and, in one study, with worse TNM staging as well [48, 49, 50]. The eighth edition of the TNM classification now includes some new changes in head and neck cancer, such as HPV/p16 in oropharyngeal cancer [9].

Overall, current evidence in the literature supports the use of serum HPV DNA as a prognostic biomarker during and after treatment, particularly for monitoring therapeutic response. Nevertheless, extrapolating from these studies to establish a practical and standardized approach to clinical monitoring is challenging, as the technologies used are not widely available in clinical settings. Further studies with extended follow-up are needed. [50, 51, 52, 53]

7. Circulating HPV Antibodies

Numerous studies have demonstrated that the presence of IgG antibodies to HPV16 is associated with a higher likelihood of HPV+ OPSCCs, supporting its etiological role [54, 55, 56]. Given that these antibodies can be detected in blood, researchers have explored their utility for non-invasive screening and monitoring. Some studies have expanded the scope of assays to include IgM, IgA, and IgG [57]. However, this study did not account for the HPV status of the tumours, limiting the ability to draw conclusions specific to HPV+ cancers. For diagnostic purposes in patients with OPSCC, E6 seropositivity has shown excellent test performance, with a sensitivity of 96% (95% CI: 88–98%) and a specificity of 98% (95% CI: 90–100%). This is comparable to commonly employed techniques such as p16 IHC [58].

The potential prognostic or monitoring role of HPV serology has also been evaluated. An early study by Rubenstein et al. found that HPV antibody titers decreased post-treatment compared to baseline in patients with primary HNSCCs. However, this analysis was not stratified by tumour site or HPV status [58].

Despite promising findings, no study has definitively addressed whether monitoring antibody levels over time could reliably predict recurrence before clinical manifestations. While antibody levels appear to hold potential as both diagnostic and prognostic biomarkers, further research is essential to establish their clinical utility.

8. Detection of HPV in Saliva

The feasibility of detecting high-risk HPV in saliva samples as a potential screening tool for HPV+ HNSCCs, as well as a non-invasive post-treatment monitoring method, has been investigated in numerous studies in the global literature.

The DNA probes used in these studies were specifically designed to amplify the E6 and E7 regions of HPV16. According to some studies, HPV in saliva was detected in 50% of patients with HPV16-positive tumour samples, 18% of patients with HPV16-negative tumour samples, and 2.7% of healthy controls [59,60]. Depending on the cut-off value for HPV copy number, the sensitivity

of the oral rinse HPV test ranged from 14% to 32%, with specificity between 97% and 99%. Overall, these results suggest that while the test is not yet suitable for broader populations, the identification of high-risk groups might be allowed for clinical application. A more recent study evaluated the diagnostic accuracy of saliva-based HPV testing in patients with known HPV+ HNSCCs [61, 62, 63].

Tumour HPV status was determined using p16 IHC and/or HPV DNA ISH on tumour specimens, and PCR amplification of 37 different HPV subtypes was performed on oral rinses. The authors reported that the oral rinse test had a sensitivity of 72.2% and a specificity of 90%, consistent with previous studies [59]. One publication reported conflicting results regarding the utility of HPV detection in saliva, though these findings may be inconclusive due to the study's small sample size [59].

Despite this, evidence suggests that HPV detection in saliva could play a role in monitoring patients diagnosed with and treated for oral carcinoma. For example, one study evaluated saliva samples from patients with HPV+ OPSCCs and found that trends in HPV DNA levels in saliva correlated with treatment response [63, 64]. Saliva samples from a cohort of 59 patients with HNSCC were analyzed at multiple time points [57, 58]. Saliva was collected before treatment, 3 months after definitive treatment, and every 3 months for a total of 2 years. Among the 59 patients, 20 patients had HPV+ SCC, and among the 20 patients with HPV-positive SCC, four developed recurrences. When quantitative PCR targeting HPV E6 and E7 was used, two of the four patients with recurrence had detectable HPV in their saliva approximately 3.5 months prior to clinical relapse. While limited by sample size, these findings suggest that saliva-based HPV monitoring may have clinical utility in patient follow-up. Similarly, in another small study of patients with HPV+ SCC, nine patients were monitored using saliva testing. Among these, three had detectable HPV in saliva before clinical recurrence, and one had HPV detected with a high likelihood of recurrence (not yet biopsy-proven at the time of publication). The remaining five patients had no detectable HPV in their saliva and did not relapse [64].

Several studies have explored the combined use of saliva and plasma for HPV DNA detection. In one study, the sensitivity and specificity of post-treatment saliva HPV16 status for predicting recurrence within 3 years were 19% and 97%, respectively, in patients with HPV16-positive tumors. The sensitivity and specificity of post-treatment plasma HPV16 status were 55% and 96%, respectively [65]. However, when post-treatment saliva and plasma HPV16 status were combined, sensitivity increased to 70%, with a specificity of 91%. Another study reported similar findings, with HPV detected in 40% of saliva samples compared to 86% of plasma samples [66].

Further studies are needed to establish a specific standardized protocol for screening OSCC and OMPD patients for HPV, as well as to define a specific and universal method.

9. HPV DNA in Saliva

Extracellular microRNAs (miRNAs) in saliva have emerged as potential diagnostic and prognostic biomarkers. However, few studies have applied this to saliva samples. Despite the promising roles of circulating microRNAs, it remains a challenge to unravel the precise regulation of these microRNAs before they can be used to target therapy [67, 68, 69].

While HPV detection in saliva is technically feasible, its role in screening, diagnosis, monitoring and prognosis is not yet well-defined and is debatable. HPV testing in saliva may also play a role in the monitoring of those patients, although larger, prospective cohort studies are needed before implementation.

Multiple methods are available for HPV detection in HNSCCs, with particular relevance for oropharyngeal tumours, where HPV status carries significant prognostic implications. The various methods for HPV detection and their comparison are challenging, as no gold standard has yet been accepted. Among these, p16 IHC for OPSCC is now routinely used in clinical practice. However, many of these methods remain in the research phase. Emerging technologies for HPV detection in saliva and serum show great promise as non-invasive tools for diagnosing and monitoring patients with oral carcinoma [70, 71].

Outlook

Various biomarkers can be used to assess the development and progression of oral carcinoma and can be used to stratify patients with regard to diagnosis and prognosis and the risk of recurrence. In assessing the accuracy of diagnostic methods for HPV detection, recent studies have highlighted reliable results from both blood and saliva samples - ctDNA, PCR, NGS. In practice, saliva-based methodologies are required, which can be easily taken in clinical settings and, given their high accuracy, can be successfully used for early detection and ongoing monitoring of patients with HPV-related malignancies. Modern advances in molecular biology in the various methods of HPV detection increase diagnostic accuracy and their integration into routine practice helps to guide treatment decisions and improve the prognosis for patients with oral carcinoma. HPV-positive and HPV-negative HNSCC have different disease profiles, but their treatment options do not yet reflect these differences. Patients with HPV-posi-

tive HNSCC generally have a better prognosis, which is due to improved tumor response to chemoradiotherapy. Both HPV-positive and HPV-negative tumors display high levels of immune checkpoint components and treatment with immune checkpoint inhibitors demonstrates higher efficacy in men than in women due to immunological differences based on gender. HPV vaccination is more effective in women, but they are also more prone to autoimmune diseases. The development of potential targeted therapies that may benefit both HPV-positive and HPV-negative HNSCC patients based on various established biomarkers is ongoing. Immunotherapy is emerging as a novel strategy for the treatment of head and neck cancer (HNSCC), especially in recurrent and metastatic tumors with very poor prognosis.

The different behavior of HPV-positive and HPV-negative head and neck squamous cell carcinoma (HNSCC) highlights the need for personalized therapeutic strategies. Advances in diagnostic technologies, noninvasive biomarker testing, and especially the use of liquid biopsies, offer promising opportunities for early detection, active surveillance, and personalized management of HPV-associated HNSCC. Further studies on the molecular mechanisms of HPV and its interactions with host cells are needed to develop targeted therapeutic approaches. Initiatives for HPV vaccination, prevention, and routine implementation of early detection methods in clinical practice are needed to effectively address this significant health problem.

CONCLUSIONS

The HPV status of oral squamous cell carcinoma lesions is considered to be an independent prognostic factor and associated with a higher 5-year survival rate and better prognosis. Early biopsy, p16 immunohistochemical staining, and HPV mRNA detection are vital for the effective treatment and prognosis determination of OSCC.

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