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Original Article

Utility of specific human papilloma virus DNA PCR to detect HPV in oral squamous cell carcinoma

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Abstract

Head and neck cancers represent the sixth most common cancer worldwide with approximately 630,000 new patients diagnosed annually resulting in more than 350,000 deaths every year.

It is essential for the understanding the biological behavior of oral squamous cell carcinomas and pre–cancerous lesions that may be responsible for malignant transformation.

Heterogeneity in prevalence and anatomic distribution are associated to demographic differences in the habits of exposure to tobacco and alcohol. It has been demonstrated that HPV infection is an independent prognostic factor, even in smoking patients, with 58% reduction of the risk of death.

The wide variation in HPV prevalence can be attributed to different detection techniques, small sample numbers, differences in the lesions and sampling techniques and epidemiological characteristics of the populations studied.

Among the many methods to detect HPV infections, polymerase chain reaction (PCR) have been well validated. HPV–positive oral squamous cell carcinomas may have a better prognosis than HPV–negative oral squamous cell carcinomas as HPV specific therapies may be available in near future. This study has been an effort to find out the incidence of HPV in oral squamous cell carcinoma in our setting.

Key words: HPV, Polymerase chain reaction, Oral squamous cell carcinoma

Introduction

Head and neck cancers rank as the sixth most common cancer globally, with approximately 630,000 new cases diagnosed each year, resulting in over 350,000 deaths annually.[1] The prevalence and anatomical distribution of these cancers vary significantly, influenced by demographic differences in tobacco and alcohol consumption habits.

While tobacco and alcohol use are well–established risk factors for oral squamous cell carcinomas, cases also occur in individuals without such exposure.[2] Human papillomavirus (HPV) infection has emerged as an independent prognostic factor, even among patients who smoke, reducing the risk of death by 58%.[3] However, the prevalence of HPV varies widely due to differences in detection techniques, sample sizes, lesion characteristics, sampling methods, and epidemiological factors across studied populations.

Various methods, including polymerase chain reaction (PCR) and immunohistochemistry (IHC), have validated HPV detection. Epidemiological studies strongly suggest that HPV, particularly type 16, plays a significant role in the pathogenesis of oral cavity and oropharyngeal squamous cell carcinomas.[4,5]

In HPV–transformed cells, the E7 protein deactivates the tumor suppressor protein RB, leading to overexpression of p16INK4a, which normally regulates cell proliferation.6 This overexpression of p16INK4a serves as a surrogate marker for HPV–transformed cells.[6,7]

HPV–positive oral squamous cell carcinomas tend to have a more favourable prognosis compared to HPV–negative counterparts, potentially due to the development of HPV–specific therapies.[8]

Materials and Methods

Study Design and Sample Collection:

A total of 30 cases of oral squamous cell carcinoma were included in this study.

The specimens were resection from the buccal mucosa (including upper and lower gingivobuccal sulcus and retromolar trigone) and tongue.

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Patients who had received adjuvant treatment prior to resection were excluded.

Demographic data including age, gender, and history of smoking were recorded for each patient.

Additionally, anatomical tumor site, pTNM stage, and histological differentiation grade were documented.

DNA Isolation and Molecular Detection of HPV:

Sample Type: Resected specimens of oral squamous cell carcinomas were fixed in 10% buffered formalin, embedded in paraffin, and stored at room temperature.

DNA Isolation Protocol was used.

Paraffin Removal was done followed by proteinase K digestion. DNA extraction was done . The extracted DNA was used for molecular detection of HPV.

Molecular Detection of HPV 16 And HPV 18 by Conventional PCR:

1. PCR amplification for HPV 16 and HPV 18 for initial molecular diagnosis of high risk HPV using the consensus degenerate primers derived from the highly conserved L1 open reading frame(ORF) of the HPV genome (with an ampliconsize of 450 bp,)
2. Typing of HPV–16 and HPV –18 was done by specific primers (synthesized from IDT) . β– globin gene was used as an internal control . The Sequence and their corresponding base pair sizes are tabulated in Table 1.

Results:

The study included a total of 30 cases of oral squamous cell carcinomas. Various sites included were buccal mucosa[10], tongue[8], gingivobuccal sulcus[12]. The age ranged from 31 to 65 years (Mean: 48 years). The mean depth of invasion was 0.45 cm (0.4 to 2.2cm). 21 were grade 2 (moderately differentiated) and 9 were grade 1 (well differentiated) squamous cell carcinoma.

HPV Detection by PCR: Out of the 30 cases analysed, 3 cases (10%) showed positive amplification of HPV DNA. The specific HPV types detected were determined if applicable (e.g., HPV type 16). Table 2 show association of T staging and nodal status with PCR results.

Discussion:

Indian subcontinent has the highest incidence of oral cavity squamous cell carcinoma in the world. Our study showed expression of HPV in 10% cases of oral squamous cell carcinoma

An Indian study of 800 cases by by Naz et al observed the HPV+ OCSCC fraction to be 13%[9].There was a significant heterogeneity in the HPV+ OCSCC prevalence worldwide, varying from 0% to 37%[10]

A systematic review of HPV prevalence in South–Central Asia resulted in the extraction of 13 studies, and almost half reported a positive association between HPV and OSCC, whereas others could not find any association. [12] A large pooled study including 3272 controls and 1157 cases reported a positive association of HR–HPV with all anatomical sites of HNSCC.[13]

Similarly, a meta–analysis presented the incidence rate of HR–HPV associated OPSCC as 24.14% [14]

S. No	Name of the Primer	Sequence	Amplicon size
1	MY09/MY11	FP: 5'–CGT CCM ARR GGA WAC TGATC–3' RP: 5'–GCM CAG GGW CAT AAY AAT GC–3'	450 bp
2	HPV–16 E6	FP: 5'–AAG GCC AAC TAA ATG TCA C–3' RP: 5'–CTG CTT TTA TAC AAC CGG–3'	217 bp
3	HPV–18 E6	FP: 5'–ACC TTA ATG AAA AAC CAC GA–3' RP: 5'–CGT CGT TTA GAG TCG TTC CTG–3'	322 bp
4	β–Globin	FP: 5'–GAA GAG CCA AGG ACA GGT AC–3' RP: 5'–CAA CTT CAT CCA CGT TAC ACC–3'	268 bp

Table 1: Primer sequences and their corresponding Base pair sizes

Case number	PCR Result		T	N	Depth of invasion
1		Negative	2	0	0.4 cm
2		Negative	2	2B	0.9 cm
3	Positive		2	3B	1 cm
4		Negative	1	0	0.6 cm
5		Negative	4	0	2.2 cm
6		Negative	3	0	2.7 cm
7		Negative	3	0	1.3 cm
8		Negative	1	1	0.4 cm
9		Negative	1	0	0.1 cm
10		Negative	3	2	1.6 cm
11		Negative	2	0	1 cm
12		Negative	4A	1	2 cm
13		Negative	2	0	0.8 cm
14	Positive		3	2B	1.4 cm
15		Negative	2	2A	0.7cm
16		Negative	2	1	0.8 cm
17		Negative	2	0	0.7cm
18		Negative	1	0	0.2 cm
19		Negative	4A	1	1.7 cm
20		Negative	4A	0	0.9 cm
21		Negative	2	0	1.3 cm
22		Negative	1	0	0.5 cm
23		Negative	3	0	1 cm
24		Negative	4A	0	2.5 cm
25		Negative	3	0	1.1 cm
26		Negative	3	0	1 cm
27		Negative	1	3B	0.5 cm
28		Negative	2	0	1cm
29		Negative	2	0	0.6 cm
30		Negative	1	0	0.5 cm

Table 2: Association of PCR result with Tumour, lymph node status and depth of invasion of oral SCC

The inconsistency of HPV detection methods across studies is observed . Various methods include PCR, ISH, HC2–HPV DNA, and p16 IHC. HPV DNA and p16 IHC lack specificity, while DNA–ISH, RNA–ISH, and E6/E7 antibody detection lack sensitivity for identifying HPV–driven

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 [11]. However, with low percentages, HR–HPV positivity is an independent risk factor for the HNSCC, especially OSCC. (11)

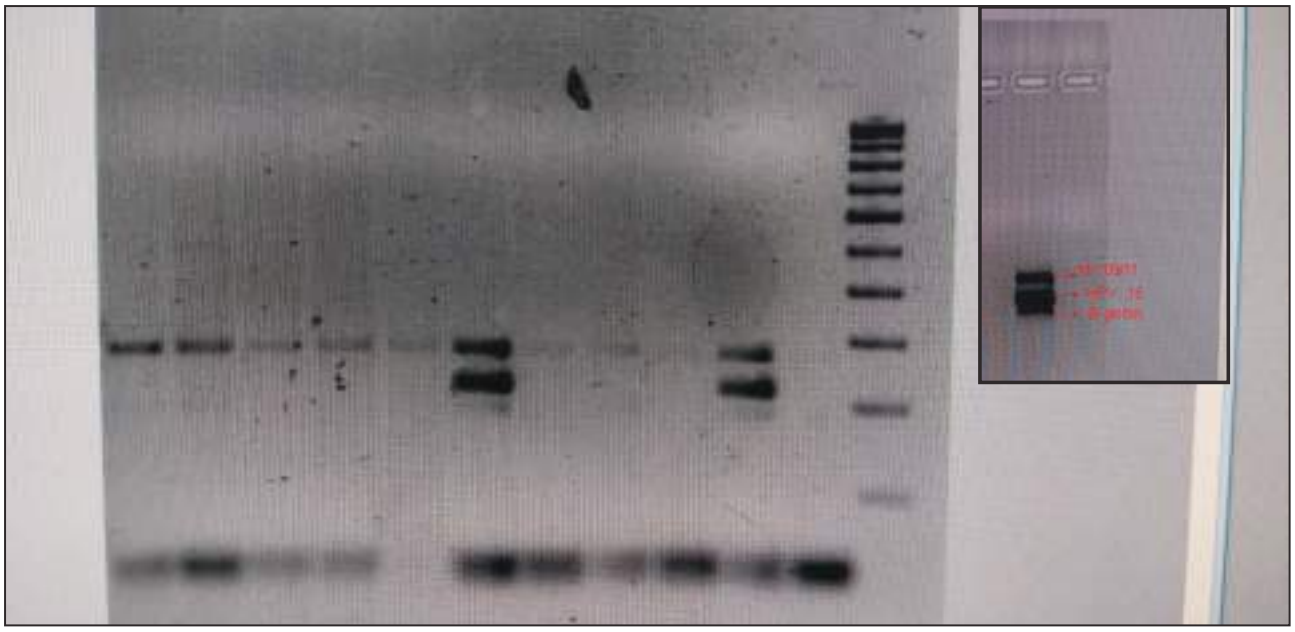


Figure 1: Gel electrophoresis of HPV16/18 and β–globin Showing lane 1–5 and 7 –9 are negative for amplification of HPV. Lane 6 and 10 show positive amplification of HPV. Lane 11–No template control. Lane 12: is a 100 bp ladder Inset shows base pair size of the amplicons

Our study aimed to evaluate the presence of HPV in OSCC using Polymerase Chain Reaction (PCR) techniques, which are renowned for their sensitivity and specificity.

HPV Prevalence in OSCC

Our findings revealed a notable prevalence of HPV in OSCC cases, aligning with previous studies that have identified HPV as a significant etiological factor in the pathogenesis of oral cancers. Specifically, our data indicate that HPV was detected in approximately 10% of the OSCC samples, consistent with the range reported in the literature.

HPV Types and Carcinogenic Potential

All the 3 cases of HPV–positive OSCC cases in our study were associated with high–risk HPV types, particularly HPV 16–. This finding corroborates existing evidence that high–risk HPV types are linked to the development of oral cancers. HPV–16 has been well–documented as a key player in the carcinogenic process due to its ability to interfere with tumor suppressor proteins like p53 and Rb.

Prognostic Implications

Some studies suggest that HPV–positive OSCC patients may have a better prognosis compared to their HPV–negative counterparts, our results did not show a significant difference in survival rates between HPV–positive and HPV–negative patients. This discrepancy could be attributed to variations in study populations, treatment modalities, or the stage of disease at diagnosis. Further longitudinal studies are necessary to clarify the prognostic value of HPV status in OSCC.

The therapeutic implications of HPV detection are also worth noting. HPV–positive OSCC may exhibit different responses to treatment compared to HPV–negative cases. For instance, some studies suggest that HPV–positive tumors may be more responsive to radiation therapy. This potential for differential treatment response emphasizes the importance of personalized treatment approaches based on HPV status.

Limitations

While our study provides valuable insights into the role of HPV in OSCC, a few limitations are acknowledged. The cross–sectional design limits our ability to assess causality and the temporal relationship between HPV infection and cancer development. Additionally, the study's sample size and geographical focus may affect the generalizability of the findings. Future research should aim to include larger, multi–center cohorts and explore the molecular mechanisms underlying HPV–driven oral carcinogenesis.

The detection of HPV in OSCC using PCR reinforces the virus's role in oral cancer etiology and highlights the potential benefits of integrating HPV status into diagnostic and therapeutic strategies.

Conclusion

HPV PCR is a valuable diagnostic tool for OSCC due to its high sensitivity and specificity in confirming HPV

involvement. However, it should be used alongside other diagnostic methods and biomarkers. With evidence linking HPV to OSCC, incorporating HPV screening into the management of high–risk patients, especially those with oral mucosal lesions, is increasingly recommended. On–going research is needed to better understand the prognostic implications and optimize management strategies for HPV–positive OSCC.

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