Short Communication

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Histopathological and Molecular Insights into Viral Oncogenesis in Oral Squamous Cell Carcinoma

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is a prevalent malignancy of the oral cavity, commonly associated with the consumption of tobacco and alcohol. However, emerging evidence also implicates oncogenic viruses in its pathogenesis. Understanding the relationship between histopathological features and viral presence is critical for improving diagnostic and treatment approaches. **Objectives:** To explore the histopathological features of OSCC and their correlation with oncogenic viruses. **Methods:** Eleven excision specimens of biopsy-proven OSCC were subjected to histopathological examination. Fresh tumor tissues, sampled intraoperatively, were sent for RTPCR-based qualitative detection of oncogenic viruses. **Results:** Nine of the eleven cases (81.8%) showed an association with oncogenic viruses. The mean age of patients was 51.4 years, with a male predominance. HPV was detected in one case; herpesviruses were found in six cases, with EBV being the most prevalent (n = 5); and non-herpes viruses were identified in four cases, with B19V being the most prevalent (n = 3). Viral co-infections were noted in three cases. All tumors were unifocal SCC, classified as Grade 1 (n = 5) or Grade 2 (n = 4). Pathologic staging revealed pT4 and pT2 in three cases each. Nodal metastasis was observed in two co-infected cases. **Conclusion:** The detection of viruses in OSCC samples emphasizes the possible role of oncogenic viruses in tumorigenesis. It also suggests that advanced stages of the disease may be associated with co-infection, highlighting the importance of viral screening. The histo-molecular correlations indicate a complex interaction between viral presence and tumor pathology, underscoring the need for studies with larger sample sizes to better interpret the mechanisms of viral oncogenesis and inform targeted interventions.

Keywords: Squamous cell carcinoma- Oral- Viruses- Histopathology

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Introduction

The buccal mucosa, a key part of the oral cavity, serves as a protective barrier against mechanical, chemical, and microbial challenges. However, it is vulnerable to a range of pathological conditions, including benign and premalignant lesions alongwith malignant transformation such as oral squamous cell carcinoma (OSCC). OSCC of the buccal mucosa is the most common malignancy in the oral cavity, known for its aggressive nature and high mortality rate. Factors such as chronic irritation, lifestyle habits, and infections contribute to this vulnerability. The global burden of OSCC remains significantly high, particularly in regions with high prevalence of alcohol consumption, betel quid chewing and tobacco use, causing lesions of the buccal mucosa. Some of these lesions, particularly those with dysplastic changes, have the potential to progress to carcinoma [1, 2].

Recent advances in research have highlighted the role of viral infections in the development of buccal mucosa lesions and OSCC. Oncogenic viruses such as high-risk human papillomaviruses (HPV), Epstein-Barr virus (EBV), and human herpesviruses (HHV) including HHV-6 and HHV-7 have been linked to these conditions, particularly in immunocompromised patients [3]. These viruses may play a role in cancer development by inducing persistent inflammation, altering immune system responses, and the expression of viral oncogenes [4]. Despite this, the extent and nature of viral involvement in OSCC remain underexplored, especially in the Indian population.

In addition to viral contributions, the interaction between host factors and environmental exposures creates a complex pathophysiological framework for OSCC.

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Understanding this interplay is essential for the screening, effective treatment approaches and prognosis. Molecular diagnostic tools, like real-time polymerase chain reaction (RT-PCR), are essential for detecting oncogenic viruses and studying the cellular behavior of affected tissues [5]. This case series aims to detect the virus and correlate with histopathological findings in OSCC, highlighting the role of viral infections and their implications in clinical outcome.

Materials and Methods

Tissue specimen collection

Approval from the Institutional Ethics Committee was obtained with reference number DYPV/EC/513/2020 for this study. Prior to sample collection, written informed consent was acquired from all the patients. Eleven patients with biopsy-proven OSCC were screened, fresh tissue biopsies from tumor were obtained intraoperatively and sent for molecular testing. The excision specimens were sent for histopathology studies.

Inclusion Criteria: All patients with biopsy-proven OSCC of age group ≥ 18.

Exclusion Criteria: Patients not willing to provide informed consent and patients belonging to the pediatric age group.

Histopathological Examination

The specimens were fixed in 10% neutral buffered formalin. Grossing was done using standard protocol and appropriate sections were taken. Tissue samples underwent automated processing using the MYR STP 120 system, involving dehydration, clearing in xylene, and paraffin infiltration. Embedded tissues were stained with hematoxylin and eosin followed by microscopic observation. Tumors were classified by World Health Organization (WHO) criteria and staged per College of American Pathologists (CAP) and American Joint Committee on Cancer Staging (AJCC) guidelines. Histopathological assessment included histological grade, perineural invasion (PNI), lymphovascular invasion (LVI), presence of underlying bone invasion, and tumor-free surgical margins. Additionally, the worst pattern of invasion (WPOI), depth of invasion (DOI), and pathological tumor (pT) stage were documented.

Screening of cells for viral etiological agents

The genomic material was extracted using the TRUPCR® Tissue DNA extraction kit (3B BlackBio Dx Limited, India) following the manufacturer's protocol. The TRUPCR® HPV High-Risk Genotyping Kit (3B BlackBio Dx Limited, India) targeting the E6/E7 region was used to identify 14 high-risk HPV genotypes 59, 18, 56, 33, 31, 35, 58, 45, 68, 52, 39, 51, 16, and 66, while the TRUPCR® Neuro Panel Kit (3B BlackBio Dx Limited, India) detected human adenovirus (HAdV), enterovirus (EV), parechovirus (PeV), herpes simplex viruses (HSV-1, HSV-2), parvovirus B19 (B19V), EBV, varicella zoster virus (VZV), cytomegalovirus (CMV), HHV-6 and HHV-7. Assays were performed as per the manufacturers' protocols on the QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA). Positive, negative, and RNaseP internal controls were used in each run. Ct thresholds were at ≤37 for HPV and ≤35 for HHV.

Results

A total of nine out of eleven cases (81.8%) showed association with oncogenic viruses, suggesting a potential viral contribution to the pathogenesis of OSCC. The clinicopathological and demographical findings of these nine cases are mentioned in Table 1. The mean age of patients was 51.4 years, ranging from 31 to 68 years. There was a strong male predominance (n=7; 77.8%) with male-to-female ratio of 7:2. Six males and one female had a history of tobacco exposure. Clinically, all the cases were diagnosed with unifocal squamous cell carcinoma (SCC) involving various parts of the oral cavity: Grade 1 (n=5) and Grade 2 (n=4). All cases had free surgical margins, indicating complete tumor resection. One case was of recurrent OSCC. Underlying bone invasion was noted in 3 cases. Pathologic stage was pT4 and pT2 in three cases each, while pT3 was seen in two cases, followed by pT1 in one case. Bone invasion, lymph node metastasis, WPOI 5, and PNI were associated with EBV and co-infections (shown in Figure 1 A to D).

RT-PCR detected HPV in only one case (HPV-51), herpesviridae viruses in six cases, and non-herpesviridae viruses in four cases, with three cases showing co-infection.

A single case showed HSV co-infection with HSV-1, EBV, and HHV-7. Also, co-infection between herpes and non-herpes viruses was noted in two cases (EBV+B19V). Nodal metastasis was seen in two cases (EBV+ B19V & EBV+HSV-1+HHV-7). WPOI 5 was associated with EBV, B19V, and HPV 51. Further results were analyzed in three groups: Herpesviridae, non-herpesviridae family, and HPV.

Herpesviridae family

The mean age was 52.66 years, with a male predominance (n=4). EBV was detected in five cases, including three with co-infection, B19V in two, and HSV-1 & HHV-7 in one case. Tumor laterality were predominantly left sided (n=5), and the buccal mucosa was the most common site (n=4). Underlying bone invasion was seen in two cases linked to EBV and HHV-6. WPOI-5 was observed in one EBV–B19V co-infection, while PNI was noted in two EBV-positive cases.

Non-herpesviridae family

Two cases showed non-herpesviridae viruses, which include HAdV & B19V. The mean age was 49 years, with all being males. The tumor laterality was right in one case while left side in another, involving the buccal mucosa and GBS. Both cases were Grade 1 SCC. Underlying bone invasion and WPOI 5 was seen in a case with B19V. None of these cases had PNI or LVI.

HPV

HPV-51 was detected in a single case of a 42-year-old male with recurrent Grade-2 SCC at the right buccal

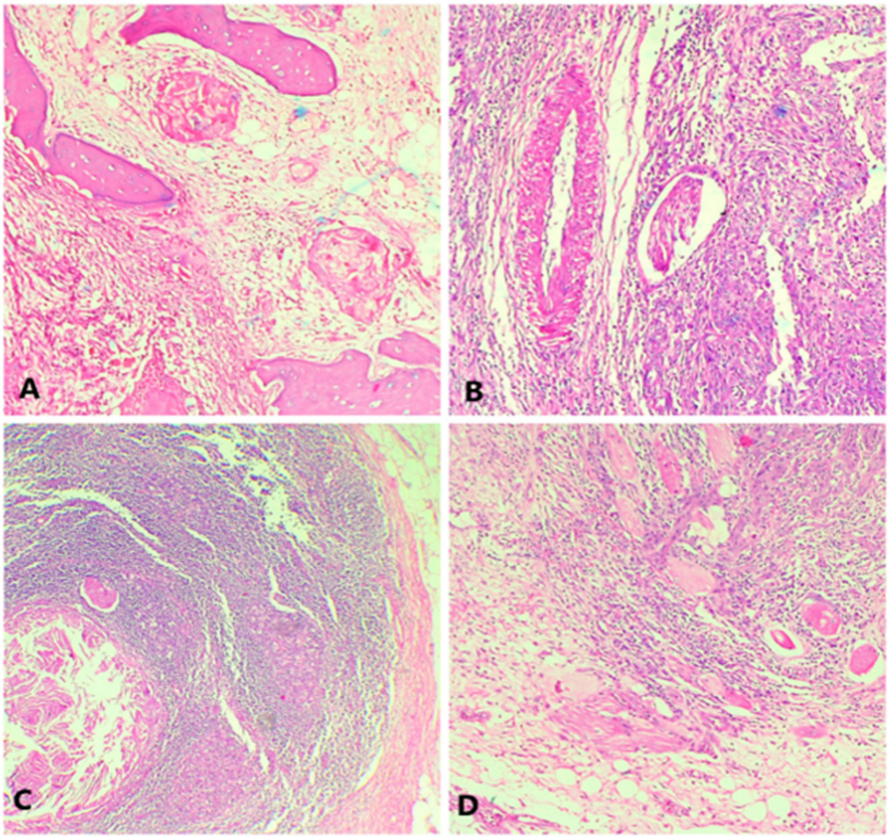

Figure 1. A, Underlying bone invasion seen in EBV & HHV-6 (H & E stain, 40X); B, Perineural invasion seen in EBV (H & E stain, 40X); C, Lymphnode metastasis seen in co-infection of EBV & other viruses (H & E stain, 40X); D,Worst pattern of invasion 5 seen in B19V & HPV-51 (H & E stain, 40X)

mucosa with WPOI 5.

Discussion

Oncogenic viruses interact with host cells to establish persistent infection, evade immune responses, and in some cases promote malignant transformation. HPV is the most prevalent virus associated with SCC of oropharynx and oral cavity. The viral oncoprotein E6 degrades p53, while E7 inactivates Rb causing disruption in DNA repair, apoptosis, triggering abnormal cell proliferation, and contributing to malignant transformation [6].

HSV-1 and HSV-2 are neurotropic viruses that establish latency in neurons and can reactivate under stress or immunosuppression. HSV-1, especially in the trigeminal ganglion, may promote oral carcinogenesis through chronic inflammation, oxidative stress, and activating oncogenes [7]. EBV infects both oropharyngeal epithelial cells and B lymphocytes. It persists latently, with its latent proteins promoting cell proliferation, inhibiting apoptosis, altering immune recognition, and contributing to oncogenesis [8]. HHV-6 infects epithelial cells, T cells, and neural tissues, whereas HHV-7 infects CD4+ T cells and salivary glands. HHV-6 is associated with oral lesions, reactivation in oral tumors and trigger the activation of herpesviruses like CMV and EBV [9]. HAdV infects mucosal epithelium, and, although mostly benign, some serotypes disrupt p53 and Rb pathways via E1A/E1B proteins. However, its role in human carcinogenesis remains speculative [10]. B19V primarily targets erythroid precursors, but its presence in oral lesions suggests a broader tissue tropism and possible immune responses or chronic inflammation [11].

Our findings identified co-infections involving EBV with B19V, HSV-1, and HHV-7. These viruses may act individually or synergistically to drive malignancy. In this case series, co-infection was linked with advanced tumor stages, including size and nodal metastasis. These findings align with previous studies suggesting that viral infections may influence the aggressiveness and metastatic potential of OSCC [12]. Co-infection was linked to poor prognostic factors, characterized by increased tumor size and the presence of nodal metastasis.

While HPV 16 and 18 are well-established contributors to head and neck cancers [13], our identification of HPV 51 in a recurrent OSCC case adds to the growing evidence that less common high-risk HPV subtypes may also play a role in oral carcinogenesis. Additionally, we observed a higher prevalence of HSV-1 over HSV-2, consistent with findings by Koivikko T et al. [14]. As per Dickinson A et al., B19V was identified in 70% of the OSCC [15], whereas our findings report B19V in 33.3% of cases. In contrast to previous studies that reported high HHV-6 positivity rates than EBV at 27.1% and 16.7%, respectively [12], our findings report a significantly higher EBV prevalence of 55.6%, whereas HHV-6 was detected in only 11.1% of the cases.

Our study has certain limitations. A key limitation of this study is the absence of a comparative control group comprising normal oral mucosa which limits

direct comparison of viral prevalence between malignant and non-malignant tissues. Additionally, due to the small sample size, robust statistical analyses could not be reliably performed, without risking type II errors. Consequently, the findings are presented descriptively that provide hypothesis-generating evidence. Another limitation is the inability to differentiate between latent and active viral infections, as PCR assays detect viral DNA but do not assess viral transcriptional or protein expression. Furthermore, serological testing and viral propagation studies were not performed, which limited a complete understanding of viral involvement and comparisons with non-cancerous tissues.

In conclusion, this case series highlights a high prevalence of oncogenic viruses in OSCC, with herpesviruses particularly EBV detected more frequently than HPV. Viral presence and co-infection were associated with adverse histopathological features, suggesting a contributory role of viral factors in tumor progression. While the current data does not indicate a role of viruses in disease pathogenesis, these findings support the concept of indirect or "hit-and-run" viral oncogenic mechanisms. Nevertheless, the results underscore the potential clinical relevance of viral screening in OSCC and provide a rationale for larger studies. Recognition of viral involvement could also inform surveillance strategies and support the future development of virus-targeted preventive, diagnostic, or therapeutic approaches.

Author Contribution Statement

Study design: A.B., M.L., and C.R. Clinical data collection: A.B., M.L., M.C., and P.P. Performed histopathological examination: A.B. and M.L. PCR assay validation and standardization: M.C., P.P. and C.R. Drafting the manuscript: A.B., M.C., P.P., and M.L. Critical revision of the manuscript: A.B., M.L., and C.R. All authors read and approved of the manuscript.

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Approval

This study was approved by college scientific committee and institution ethics commitee of Dr. D.Y. Patil Medical College, Hospital and Research Centre, ensuring compliance with ethical standards and guidelines.

Ethical Declaration

This study protocol was reviewed and approved by the Institutional Ethics Committee (reference number-DYPV/EC/513/2020) of Dr. D. Y. Patil Medical College, Hospital and Research Centre, Pimpri, Pune, Maharashtra, India. Written informed consent was obtained from all patients (or their parent/legal guardian/next of kin) to participate in the study.

Table 1. Clinicopathological and Demographical Findings of OSCC Samples Showing Association with Oncogenic Viruses

Sr.No	Age	Sex	Clinical diagnosis	Procedure	Site Of Tumor	Laterality	Grade	WPOI	LVI	PNI	Stage	HPV PCR	HSV PCR	NON- HSV PCR
1	42	M	Recurrence of SCC	Right maxillectomy with hemi mandibulectomy	Buccal mucosa	Right	G2	5	NI	NI	rpT3	HPV-51	Negative	Negative
2	54	M	CA left buccal mucosa	Wide local excision of left buccal mucosa	Buccal mucosa	Left	G1	3	NI	NI	pT1pN	Negative	Negative	HAdV
3	59	M	Left cheek SCC	Wide local excision of left buccal mucosa with marginal mandibulectomy	Buccal mucosa	Left	G1	3	NI	NI	pT3pN0	Negative	EBV	B19V
4	66	F	SCC of upper alveolus	Right infrastructure maxillectomy	Upper lateral alveolar ridge	Right	G1	3	NI	NI	pT4apNo	Negative	HHV- 6	Negative
5	31	M	SCC of GBS	Wide local excision of left buccal mucosa	Left upper GBS	Left	G2	3	+	+	pT2pN1	Negative	HSV- 1, EBV & HHV- 7	Negative
6	60	F	SCC of GBS	Left segmental mandibulectomy	Left lower GBS	Left	G2	3	NI	+	pT4apN0	Negative	EBV	Negative
7	51	M	SCC	Right hemi mandibulectomy	Retromolar trigone involving GBS	Right	G1	5	NI	NI	pT4bN0PM	Negative	Negative	B19V
8	54	M	SCC of left mandibular alveolus	Left segmental mandibulectomy	GBS	Left	G1	5	NI	NI	pT2bN2b	Negative	EBV	B19V
9	46	M	SCC of tongue	Left hemiglossectomy	Lateral border of tongue	Left	G2	3	NI	NI	pT2bN0	Negative	EBV	Negative

*FOOTNOTE, WPOI-Worst pattern of invasion; LVI, Lymphovascular invasion; PNI, Perineural invasion; HPV, Human papillomavirus; HSV, Herpes simplex virus; SCC, Squamous cell carcinoma; CA, Cancer; GBS, Gingivobuccal sulcus; NI, Not identified, +, Present

Data Availability

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

Conflict of Interest

The authors have no conflicts of interest to declare.

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