Journal of Chemical Health Risks

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JCHR (2025) 15(1), 341-347 | ISSN:2251-6727

A Study of Squamous Cell Carcinoma in Upper Aerodigestive Tract and Its Association with Hpv

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(Received: 16 November 2024 Revised: 11 December 2024 Accepted: 11 January 2025)

KEYWORDS
Squamous cell carcinoma, upper aerodigestive tract, HPV, p16 immunohistochemistry.

ABSTRACT:

Introduction: Squamous cell carcinoma (SCC) of the upper aerodigestive tract (UADT) is one of the most prevalent malignancy worldwide with tobacco use and human papillomavirus (HPV) infection recognized as major risk factors. This study aimed to evaluate the prevalence of HPV in SCC cases and assess its association with clinicopathological parameters in a tertiary care hospital.

Methodology: A cross-sectional study was conducted on 150 biopsy-confirmed SCC cases, with p16 immunohistochemistry (IHC) used as a surrogate marker for HPV detection. Data were analysed using SPSS version 20.

Results: The majority of participants (76%) were above 40 years of age and 76% were male. Tobacco consumption was prevalentwith 78% having a history of tobacco use. Site-wise analysis revealed that the oral cavity accounted for most cases (n=90) with 22% testing positive for p16 indicating HPV association. The upper oesophagus had the p16 positivity 16% despite fewer cases, while sites such as the larynx, oropharynx, and hypopharynx showed lower positivity rates. Histopathologically, 43.3% of cases were moderately differentiated SCC, followed by 30% well-differentiated, 23.3% poorly differentiated, and 3.3% de-differentiated SCC. Overall, p16 positivity was observed in 16% of cases, highlighting a lower prevalence of HPV in this cohort.

Conclusion: SCC of the UADT in this study was predominantly associated with older males and tobacco use with HPV playing a limited role compared to western populations. Site-specific HPV variations emphasize the need for targeted screening and management strategies. Further research into molecular profiling and larger cohorts is necessary to validate these findings and refine therapeutic interventions.

INTRODUCTION:

Cancers of the upper aerodigestive tract (UADT) rank as the fifth most common malignancy globally. Among these, squamous cell carcinoma (SCC) is the predominant histological variant accounting for approximately 90% of cases. In India, oral cancer represents nearly 30% of all cancer cases.[(1)]

The UADT encompasses structures including the nasal cavity, paranasal sinuses, nasopharynx, oral cavity, oropharynx, hypopharynx, larynx, trachea and oesophagus.[(2)]SCC of the UADT is a broad and diverse

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subject of study. Within this region, the oral cavity and larynx are the most frequently affected sites, whereas the oesophagus and sinonasal regions are less commonly involved. HPV-positive SCC, particularly those associated with high-risk subtypes HPV 16 and 18 generally exhibit a more favourable prognosis compared to HPV-negative cases.[3]Tobacco abuse is another well-established risk factor for SCC development.

Human papillomaviruses (HPV) are small, non-enveloped, icosahedral DNA viruses belonging to the Papovaviridae family. The viral genome contains early (E1-E7) and late (L1-L2) genes. The E6 protein of HPV binds to and inactivates the p53 tumour suppressor protein, while the E7 protein disrupts the retinoblastoma protein (pRb). This interference deregulates the cell cycle, promoting uncontrolled cellular proliferation.[4]

p16 is frequently inactivated in oral SCC. However, in HPV-associated cancers, such as oral cavity and oropharyngeal SCC, p16 is overexpressed via a negative feedback mechanism. Immunohistochemical (IHC) analysis has shown p16 positivity primarily in Grade 3 and Grade 4tumours. Hypermethylation is one of the most common mechanisms leading to p16 inactivation.[5]

Detection of HPV expression using p16 is regarded as the gold standard for evaluating HPV-associated SCC. A meta-analysis indicated that the pooled prevalence of HPV infection in SCC cases was approximately 33% (95% CI: 25.8–42.6) with substantial heterogeneity (I^2 = 95%). Geographical variations were also noted with higher prevalence rates in eastern regions (47%; 95% CI: 32.2–62.4) compared to western regions (19.8%; 95% CI: 10.8–33.4).[6] Globally, head and neck cancers account for over half a million new cases annually, positioning them as the fifth most common malignancy worldwide.[7]

This study aims to evaluate squamous cell carcinoma of the upper aerodigestive tract and investigate its association with Human papillomavirus (HPV) in a tertiary care hospital. The primary objective is to detect the presence of Human papillomavirus in squamous cell carcinoma of the upper aerodigestive tract using p16 Immunohistochemistry markers. Additionally, this study aims to assess the demographic parameters and pathological distribution of squamous cell carcinoma within the upper aerodigestive tract. Finally, it seeks to determine the prevalence of Human papillomavirus in cases of squamous cell carcinoma of the upper aerodigestive tract in patients treated at a tertiary care hospital. Through these objectives, the study aims to provide valuable insights into the role of HPV in the pathogenesis of squamous cell carcinoma and identify patterns that could assist in better diagnosis, management, and treatment planning.

MATERIALS AND METHODS:

Study Design: This is a cross-sectional study designed to evaluate the association between HPV infection and squamous cell carcinoma of the upper aerodigestive tract. The study involves histopathological and immunohistochemical evaluations to detect p16 expression, which serves as a surrogate marker for HPV infection.

Study Location: The study is conducted in the Department of Pathology, Bharati Vidyapeeth (Deemed to be University), Medical College and Hospital, Sangli, which caters to a diverse population, ensuring a representative sample of SCC cases.

Study Duration: The study spans one year from March 2023 to February 2024, allowing sufficient time for sample collection, processing and analysis.

Study Subjects: The study includes biopsy tissue specimens obtained from patients diagnosed with upper aerodigestive tract squamous cell carcinoma confirmed through histopathological examination. Biopsy specimen including small incisional biopsies and radical primary surgeryincluding neck dissection specimens are included.

Inclusion Criteria:

- Patients with histopathologically confirmed SCC of the upper aerodigestive tract.
- Patients who have undergone radical primary surgery, including neck dissection as well as small incisional biopsies.

Exclusion Criteria:

- Biopsy samples that are inadequate or lack sufficient tissue for immunohistochemical analysis.

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- **Sample Size:** A total of 150 biopsy tissue specimens from histologically confirmed oral squamous cell carcinoma (OSCC) patients were included.

Data Collection: Demographic details, clinical history and histopathological findings were recorded. Tissue samples were fixed in formalin, embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) staining. Immunohistochemical analysis was conducted using p16 antibody markers to detect HPV-associated cases.

Laboratory Methods: Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections. Antigen retrieval was carried out, followed by incubation with primary anti-p16 antibodies. Visualization was achieved using a DAB (diaminobenzidine) chromogen, and counterstaining was performed with hematoxylin. Positive p16 expression was indicated by blockstaining- strong nuclear and cytoplasmic expression in a continuous segment of cells (at least 10 - 20 cells)in squamous epithelium.

Statistical Methods: Data were analyzed using SPSS software (version 20). Descriptive statistics were used to summarize demographic and clinical data. Chi-square tests were employed to evaluate associations between HPV positivity and clinical parameters. Statistical significance was set at $p < 0.05$.

Ethical Considerations: The study was approved by the institutional ethics committee. Informed consent was obtained from all participants prior to inclusion in the study.

RESULTS:

Socio-Demographic Characteristics: The majority of patients were above 40 years of age, accounting for 76% (n=114) of the cases. Patients in the 20–40 years age group comprised 21.3% (n=32), while those below 20 years constituted only 2.7% (n=4). This suggests that squamous cell carcinoma of the upper aerodigestive tract is more prevalent in older individuals. (Table 1)

Male patients were more commonly affected comprising 76% (n=114) of the total cases, whereas females accounted for 24% (n=36). This indicates a significant male predominance in the incidence of SCC in the study population. (Figure 1)

Site wise distributionof SCC and HPV:The distribution of p16 positivity across different anatomical sites is presented in Table 2. The highest number of cases was observed in the oral cavity (n=90) with 22% testing positive for p16. The larynx had 14 cases, of which only 7.1% showed p16 positivity. In the oropharynx, 24 cases were documented, with 4.1% being p16 positive. Hypopharynx cases (n=12) exhibited an 8.3% positivity rate, while the nasopharynx (n=4) showed no p16 positivity. Interestingly, the upper oesophagus, with only 6cases, demonstrated the highest p16 positivity at 1. (Table 2)

Habit Distribution: Tobacco consumption was highly prevalent among the study participants. A combination of tobacco chewing and smoking was observed in 78% (n=117) of the patients, while 22% (n=33) were exclusive smokers. This highlights the strong association between tobacco habits and the development of SCC. (Table 3)

Histopathological Findings: Table 4 presents the distribution of study participants according to their histopathological impression. The majority of cases, 43.3% (n=65), were moderately differentiated squamous cell carcinoma (Grade 2). This was followed by well-differentiated squamous cell carcinoma (Grade 1), comprising 30% (n=45) of cases. Poorly differentiated squamous cell carcinoma (Grade 3) accounted for 23.3% (n=35), while only 3.3% (n=5) of cases were classified as de-differentiated squamous cell carcinoma (Grade 4). These findings suggest that most cases were in the moderately differentiated category, indicating a relatively intermediate grade of malignancy. The presence of 23.3% poorly differentiated and 3.3% de-differentiated cases highlights a subset of more aggressive tumours with potentially worse prognoses. (Figure 2-5)

Immunohistochemistry (IHC) Results: Analysis of p16 expression revealed that 84% (n=126) of the cases were immunonegative for p16, while 16% (n=24) were immunopositive. This finding suggests a lower prevalence of HPV-associated SCC in this cohort, indicating that the majority of cases may be unrelated to HPV infection. (Table 5)

The distribution of cases according to anatomical sites is presented Table 2. The majority of cases were found in the oral cavity constituting the largest proportion. Other

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affected sites included the oropharynx, larynx, hypopharynx and nasopharynxin decreasing order of frequency. This highlights the oral cavity as the most common site for SCC involvement in this study.

The results collectively highlight that SCC of the upper aerodigestive tract is predominantly observed in older males with a history of tobacco use. The low proportion of HPV positivity suggests that tobacco-related factors may play a more significant role in this population, with HPV acting as a contributing factor in a minority of cases.

Table 1: Age distribution of the study participants

Age	No. of patients	Percent
<20 years	4	2.7
20-40 years	32	21.3
>40 years	114	76
Total	150	100

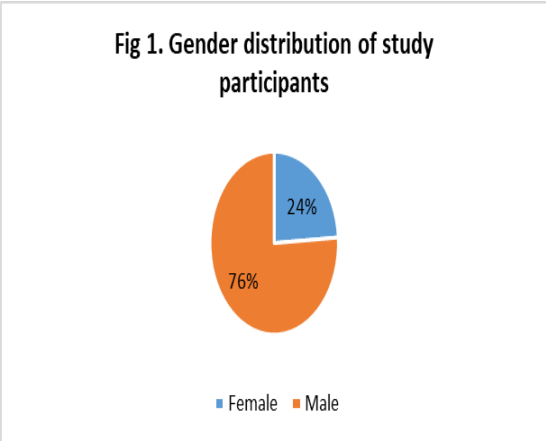

Table 2: Distribution of study participants according to their site

Site	Number of cases (n)	p16 positive on IHC	Percent
Oral cavity	90	20	22
Larynx	14	01	7.1
Oropharynx	24	01	4.1
Hypopharynx	12	01	8.3
Nasopharynx	04	00	0
Upper oesophagus	06	01	16

Table 3: Distribution of study participants according to their habits

Habit	Frequency	Percent
Chewing tobacco + Smoker	117	78
Smoking tobacco	33	22
Total	150	100

Table 4: Distribution of study participants according to their Histopathological impression

Histopathological Impression	Frequency	Percent
Well differentiated Squamous cell carcinoma (Grade 1)	45	30
Moderately differentiated Squamous cell carcinoma (Grade 2)	65	43.3
Poorly differentiated Squamous cell carcinoma (Grade 3)	35	23.3
De-differentiated Squamous cell carcinoma (Grade 4)	05	3.3
Total	150	100

Table 5: Distribution of study participants according to their IHC impression

IHC impression	Frequency	Percent
Immunonegative for p16	126	84
Immunopositive for p16	24	16
Total	150	100

Figure No.2: Moderately differentiated SCCshowing atypical squamous cell proliferation in cords and nest infiltrating the dermis with focal individual keratinization (40X magnification on H& E stain).

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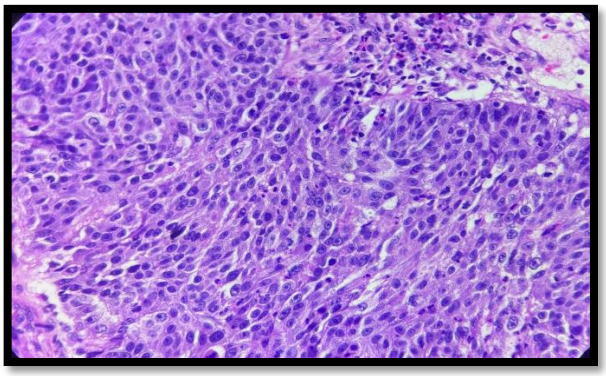


Figure No.3: Well- differentiated SCC (40x magnification H & E stain) showing atypical squamous cell proliferationinfiltrating the dermis with central keratinization.

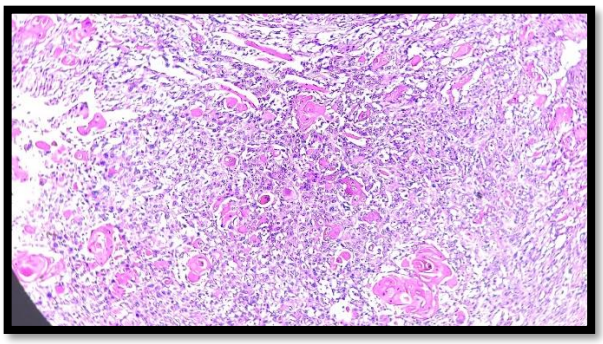
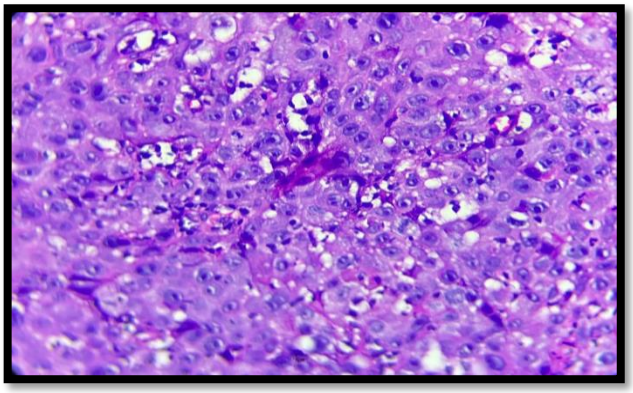
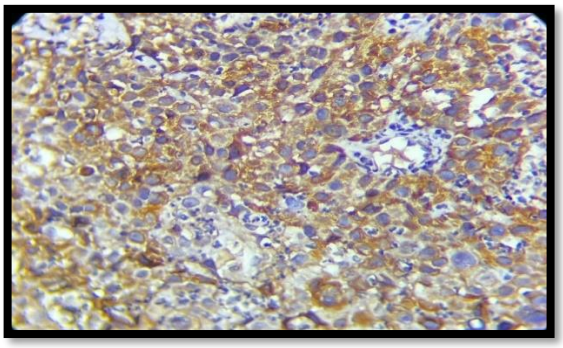


Figure No.4: (A) Moderately SCC on H &E stain (40X magnification) & (B) p16 IHC stain (40X magnification) showing block positivity

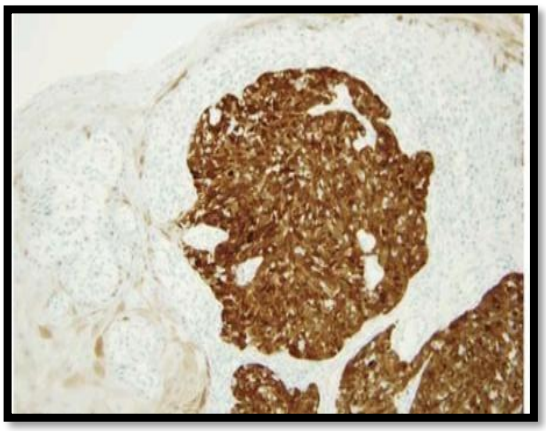


(A)



(B)

Figure No.5: p16 IHC stain (40X magnification) showing block positivity of the tumour cells.



DISCUSSION:

The findings of this study highlight a predominantly older male population affected by SCC of the upper aerodigestive tract, with a high prevalence of tobacco use and a low incidence of HPV positivity. This aligns with the study by Hashmi et al., who reported p16 positivity in only 22.9% of cases and suggested a low prevalence of HPV-driven SCC in their cohort.[8]Similarly, Dhar et al. observed a 29% HPV positivity rate in SCC cases and emphasized tobacco and alcohol as primary etiological factors.[9] In contrast, Giuliano et al. noted higher HPV prevalence rates, particularly in oropharyngeal cancers, which may reflect differences in regional exposure and sexual behaviors.[10]

Site-wise distribution of SCC and HPV it was seen the predominance of cases in the oral cavity with 22 % p16 positivity that aligns with findings by Hashmi et al., who reported higher HPV association in oral cancers

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compared to other anatomical sites.[8] This observation highlights the oral cavity as a common site for HPV-driven carcinogenesis. However, the lower p16 positivity in the larynx (7.1%) and oropharynx (4.1%) parallels findings by Kim et al., who emphasized anatomical variations in HPV prevalence.[11]The hypopharynxwith an 8.3% positivity rate showed moderate HPV association, comparable to findings by Mehanna et al., who reported similar rates in non-oropharyngeal sites.[5]The absence of p16 positivity in the nasopharynx may reflect its distinct etiology, often associated with Epstein-Barr Virus rather than HPV, as supported by Beck et al.[12]Interestingly, the upper esophagus demonstrated the p16 positivity at 16 %, consistent with Dhar et al., who highlighted the potential role of HPV in esophageal SCC.[9]These variations show the importance of site-specific investigations to better understand the role of HPV in SCC and guide targeted screening and management strategies.

Kim et al. reported that HPV positivity was more frequent in tonsillar SCC than in other UADT sites, reinforcing the anatomical variation in HPV association.[11]Chernock et al. also identified HPV-driven basaloid SCC as a distinct subset with better prognostic outcomes.[13] These findings contrast with the present study, where p16 positivity was low, possibly due to differences in sample size, anatomical focus, and testing methodologies.

Differences in methodology may also explain variability. For example, Anwar et al. used PCR-based detection and observed a 3.8% prevalence of HPV, which is comparable to this study's findings.(14) Variations in demographic factors, such as age and gender distribution, may further influence HPV prevalence. Beck et al. highlighted that HPV-positive cases often occurred in younger populations, whereas HPV-negative cases were more common in older individuals.[12]

The predominance of tobacco use in this cohort underscores its role as a major risk factor. This is consistent with studies by Dhar et al. and Kim et al., who emphasized tobacco and alcohol as synergistic carcinogens.[9,11] Furthermore, p16-negative tumours in this study may have a poorer prognosis compared to HPV-positive cases, as reported by Mehanna et al.[5]

Overall, the low prevalence of HPV in this cohort highlights the need for region-specific data to guide targeted interventions. Future studies should focus on larger sample sizes, molecular profiling, and longitudinal follow-ups to better understand the role of HPV in SCC of the UADT.

CONCLUSION:

This study highlights that squamous cell carcinoma of the upper aerodigestive tract predominantly affects older males with a strong history of tobacco use, reinforcing tobacco as a key risk factor. HPV positivity was relatively low, indicating that HPV may play a limited role in this region compared to other geographical areas where HPV-driven SCCs are more prevalent. The findings emphasize the need for region-specific screening strategies, with a focus on tobacco cessation programs, while further exploring the role of HPV in SCC pathogenesis. Future research should incorporate molecular profiling and larger sample sizes to validate these findings and improve targeted therapeutic interventions.

REFERENCES:

1. Murthy V, Calcuttawala A, Chadha K, d'Cruz A, Krishnamurthy A, Mallick I, et al. Human papillomavirus in head and neck cancer in India: Current status and consensus recommendations. South Asian J Cancer. 2017 Jul;06(03):093–8.
2. Perri F, Longo F, Caponigro F, Sandomenico F, Guida A, Della Vittoria Scarpati G, et al. Management of HPV-Related Squamous Cell Carcinoma of the Head and Neck: Pitfalls and Caveat. Cancers. 2020 Apr 15;12(4):975.
3. Raya GVV, Manjunath G V. To study HPV and EBV in squamous cell carcinoma of upper aerodigestive tract by immunohistochemistry markers. Indian J Pathol Oncol. 2022 May 28;9(2):145–52.
4. Tomaić V. Functional Roles of E6 and E7 Oncoproteins in HPV-Induced Malignancies at Diverse Anatomical Sites. Cancers. 2016 Oct 19;8(10):95.
5. Mehanna H, Taberna M, Von Buchwald C, Tous S, Brooks J, Mena M, et al. Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational,

individual patient data analysis. Lancet Oncol. 2023 Mar;24(3):239–51.

6. Satapathy P, Khatib MN, Gaidhane S, Zahiruddin QS, Serhan HA, Sharma RK, et al. Prevalence of human papillomavirus in head and neck cancer patients in India: a systematic review and meta-analysis. BMC Infect Dis. 2024 May 23;24(1):516.
7. Wenig BM. Squamous cell carcinoma of the upper aerodigestive tract: dysplasia and select variants. Mod Pathol. 2017 Jan;30:S112–28.
8. Hashmi AA, Younus N, Naz S, Irfan M, Hussain Z, Shaikh ST, et al. p16 Immunohistochemical Expression in Head and Neck Squamous Cell Carcinoma: Association With Prognostic Parameters. Cureus [Internet]. 2020 Jun 13 [cited 2024 Dec 22]; Available from: https://www.cureus.com/articles/16883-p16-immunohistochemical-expression-in-head-and-neck-squamous-cell-carcinoma-association-with-prognostic-parameters
9. Dhar L, Singh S, Passey JC. Association of human papillomavirus and Epstein–Barr virus with squamous cell carcinoma of upper aerodigestive tract. Natl J Maxillofac Surg. 2022 Sep;13(3):367–75.
10. Giuliano AR, Felsher M, Waterboer T, Mirghani H, Mehanna H, Roberts C, et al. Oral Human Papillomavirus Prevalence and Genotyping Among a Healthy Adult Population in the US. JAMA Otolaryngol Neck Surg. 2023 Sep 1;149(9):783.
11. Kim KH, Kabir E, Kabir S. A review on the human health impact of airborne particulate matter. Environ Int. 2015 Jan 1;74:136–43.
12. Beck TN, Smith CH, Flieder DB, Galloway TJ, Ridge JA, Golemis EA, et al. Head and neck squamous cell carcinoma: Ambiguous human papillomavirus status, elevated p16, and deleted retinoblastoma 1. Head Neck [Internet]. 2017 Mar [cited 2024 Dec 22];39(3). Available from: https://onlinelibrary.wiley.com/doi/10.1002/hed.24604
13. Chernock RD, Lewis JS, Zhang Q, El-Mofty SK. Human papillomavirus–positive basaloid squamous cell carcinomas of the upper aerodigestive tract: a distinct clinicopathologic and molecular subtype of basaloid squamous cell carcinoma. Hum Pathol. 2010 Jul;41(7):1016–23.
14. Anwar N, Chundriger Q, Awan S, Moatter T, Ali TS, Abdul Rasheed M, et al. Prevalence of high-risk human papillomavirus in oral squamous cell carcinoma with or without chewing habits. Langevin SM, editor. PLOS ONE. 2024 May 1;19(5):e0300354.