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P16INK4A Expression in Young Adults with Oral Squamous Cell Carcinoma: Evaluating HPV Etiology and Impact of Deleterious Oral Habits.

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Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is a significant health concern, particularly in India, where it accounts for 40% of all cancers. OSCC in individuals under 40 years forms a distinct subset, often without traditional risk factors like tobacco or alcohol use. Human papillomavirus (HPV)-related oncogenesis is increasingly recognized in this demographic. This study evaluated p16 immunohistochemical expression, a surrogate marker for HPV infection, in young OSCC patients with and without deleterious habits.

Materials and Methods: Archival records from 2016–2023 were analyzed for 495 OSCC cases, detailing demographic, clinical, and histopathological characteristics. Forty cases under 40 years were selected and divided into two groups: Group I (with habits, n=20) and Group II (non-habituates, n=20). Immunohistochemical p16 expression was assessed and categorized based on positivity percentages. Statistical analysis was performed using the t-test.

Results: Group II showed significantly higher p16 expression (59.5%) than Group I (40.4%) ($p<0.03$). HPV-driven pathogenesis was more prevalent in non-habituates, particularly affecting the anterior tongue and gingiva. Among 495 OSCC cases, 10.5% were under 40 years, with a male predominance (75%). The tongue (58.4%) and buccal mucosa (39.6%) were the most affected sites. Histopathologically, 75.5% were moderately differentiated, and 92.4% had deleterious habits. Among patients under 40, 68.5% were habituates, with habits more prevalent among males (87.8%, $p<0.012$).

Conclusion: Young non-habituate OSCC cases exhibit distinct molecular profiles, highlighting HPV's role. Incorporating p16 analysis into diagnostics can improve identification and enable personalized treatment approaches.

Keywords: human papilloma virus, immunohistochemistry, oral squamous cell carcinoma, p16, young adults

Introduction

According to Global Cancer Observatory2022, OSCC of lip & oral cavity ranks 16th& carcinoma of oropharynx 24th among all cancers worldwide. Habits such as tobacco use, alcohol intake, and smoking are the leading causes of OSCC, accounting for at least 80% of OSCC [1] . Despite the growing awareness about the benefits of quitting tobacco, smoking, and drinking alcohol, the number of occurrences of oral malignancies have been increasing lately[2,3,4] . Also, recent epidemiological data show a concerning rise in incidence among individuals aged 15-39 years. This shift in epidemiology has been attributed to Human Papilloma Virus (HPV) infection [5].

International agency for research on cancer in 2022, considered high-risk types of human papillomavirus (HPV), an extremely common family of viruses, as a potent carcinogen. p16INK4A, a cyclin-dependent kinase inhibitor, is commonly used as an immunohistochemical (IHC) surrogate marker for high-risk HPV infection [6]. According to the AJCC (8th edition) guidelines, p16 IHC should be done in all oropharyngeal cancers with a separate staging system for HPV positive OPSCC [7]. According to recent studies across Asian countries, it is found that South Asia had the highest prevalence (27.1%), followed by East Asia (19.4%), and Southeast Asia (16.7%). Anatomical subsites, buccal mucosa (34.0%), and floor of the mouth (33.2%) had similar ranges of HPV prevalence [8]. However, the prevalence in India was 28.43%, which is slightly higher than the global prevalence [9]. The published literature regarding prevalence of HPV in Kerala is limited, recent one being conducted by Raj D et al in 2024, according to which the prevalence of HPV 16 was 6.6% and all were carcinoma tongue [10]. So in this study we aimed to evaluate the expression of p16 in OSCC among young adults using immunohistochemistry and correlate it with clinicopathological parameters in our settings.

Materials &Methods

The archival records of patients reported to the Department of Oral Pathology and Microbiology, between 2017 and 2023 were retrospectively reviewed.495 OSCC cases were analysed, detailing demographic, clinical, and histopathological characteristics. The data extracted were analyzed to determine its distribution according to age, gender, site, habits and histopathologic types. The anatomical sites reviewed in this study included buccal mucosa, alveolus, hard palate, tongue and floor of mouth (FOM). Descriptive statistics were used for frequency and percentage calculations.

For IHC, a cross sectional study was done. Forty cases under 40 years were selected and divided into two groups: Group I (with habits, n=20) and Group II (non-habituates, n=20). Immunohistochemical analysis of p16INK4A was performed on 40 formalin – fixed and paraffin – embedded samples of OSCC. Staining was measured by percentage of cells taking up the stain in 40x magnification. Immunohistochemical p16 expression was assessed and categorized based on positivity percentages. Statistical analysis was performed using the t-test.

Results

Among the study participants total of 495 patients with OSCC in the 6-year period from 2017 to 2023 were retrospectively analyzed in the present study. Among this, patients 442 (89.49%) were above 40 years and 52 patients (10.5%) were below the age of 40 years. A significantly high male preponderance was noted with 75% of total cases. The mean age of presentation for males was 70.3 years and for females 68.6 years. A site predilection was noted for tongue with171 in males (48%), 55 in females followed by buccal mucosa in 104 males (28%) and 49 in females, alveolus in 52males (13.9%) and 5in females, palate in 27 males (7.07%) and 3 females and FOM in 18 males (4.84%) and 11females. Histopathological examination showed that among these, 374 (75.5%) were moderately-differentiated tumors, 102 (20.6%) were well differentiated, and 19(3.8%) were poorly differentiated tumors. 92.4% of patients were having deleterious habits and 7.6% of patients were non habituate.

On detailed analysis done in patients younger than 40 years, and it was found that there were a significant risk number of OSCC cases in this age group. A male sex predilection (75%) which was comparable to that of total cases was noted. In cases below 40 years of age, the mean age at presentation was 39.1 years for males and for females 34.25 years (P=0.436). For patients below 40 years of age, more cases were seen in tongue (58.4%) followed by buccal mucosa (39.6%) and palate (1.98%). Males show a site predilection for tongue with 23 cases (56%) followed by buccal mucosa 17 cases (41.4%) and palate 1 (0.02%).No cases were reported to occur in FOM and alveolus. In females more cases were seen in tongue (63.6%) and buccal mucosa (36.3%). Histopathological grading also shows distribution with MDSCC (66.3%), WDSCC (32.6%) and PDSCC (0.9%) in males and MDSCC (63.6%) and WDSCC (36.3%) in females. Among the cases, 68.5% of patients had deleterious habits with a male dominance (87.8%). Rests of the patients were non habituate with a female predilection of 54.2%. Considering habit as a risk factor for OSCC among males and females below 40 years of age, it was found that, the association of habit with OSCC in

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males is significant than the association in females with a p value of <0.012.

Immunohistochemical analysis of p16INK4A was performed on formalin – fixed and paraffin – embedded samples. Cervical carcinoma tissue was taken as positive control and normal oral mucosa as negative control. It is then measured by percentage of cells taking up the stain in 40x magnification. Data statistically analysed using t test.

Table.1: Mean p16 expression:

Deleterious habits	N	P16 expression		t	p
		Mean	SS		
yes	20	0.95	8.95	2.21	<.033
no	20	1.4	6.8		

Average expression score of p16 protein in Patients with habits was 0.95 (lower expression) and Patients without habits has a mean expression of 1.4 (higher expression). Higher t-value suggests a greater difference between the means relative to variation. P < 0.033 which indicates there is a statistically significant difference between the two groups. In conclusion patients without deleterious habits had significantly higher p16 expression than those with habits.

Table 2: Percentage distribution of variables among OSCC cases in the period of 2017 – 2023

Age	**<40 years – 52 (10.5%)** **>40years – 441 (89.09%)**
Gender Male	**372 (75%)**

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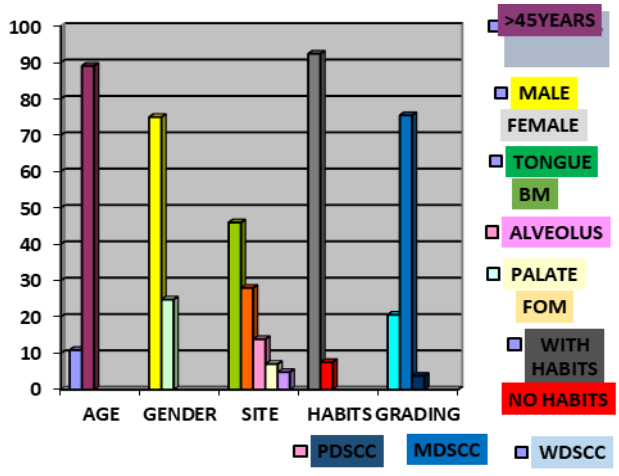
Female	**123 (24.8%)**	
Site	Male	Female
Tongue	171(46%)	
Buccal mucosa	55(44.7%)	
Alveolar ridge	104 (28%)	
Palate	49(39.8%)	
Floor of the mouth	52 (13.9%)	5(4.07%)
	27(7.07%)	3(2.43%)
	18 (4.84%)	11(8.9%)
Grading	102 (20.6%)	
WDSCC	374(75.5%)	
MDSCC	19(3.8%)	
PDSCC		
Habit	457(92.4%)	
With deleterious habits	38 (7.6%)	
Without deleterious habits		

Graph 1.Graphical representation of distribution of variables among OSCC reported in the period of 2017 – 2023



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Discussion

In recent years, data suggest that there has been a notable rise in number of oral squamous cell carcinoma cases in individuals without any deleterious habits. The incidence of lip and oral cavity cancer in 2022, as reported by the Global Cancer Statistics (GLOBOCAN), ranking 16th globally, amounted to 389,846 cases, with an age-standardized rate (ASR) of 4.0. It ranked 15th in mortality, with 188,438 deaths and an ASR of 1.9. Recent research suggests that 4-6% of oral cancers in India are not associated with any deleterious oral habits[11] This changing pattern suggests the involvement of additional etiological factors, including human papillomavirus (HPV) infection, chronic irritation due to trauma and genetic susceptibility. Unlike older patients, who typically have a long history of tobacco or alcohol exposure, many younger individuals develop OSCC in the absence of these conventional risk factors.In our study we aim to evaluate the clinicopathological features and p16 expression amongyoung adults without any deleterious habits.

HPV is a group of DNA viruses which forms a separate Papovaviridae family, which includes Papillomaviridae and polyomaviruses[12,13].More than 200 different human papillomaviruses (HPVs) have been listed so far. Based on epidemiological data, a subgroup of alphapapillomaviruses (alpha HPVs) was referred to as high-risk (HR) HPV types [14]. HPV is also classified according to cutaneous or mucosal tropism characteristics. The cutaneous types, mainly from beta and gamma genera, are associated with skin lesions; mucosal types infect the anogenital tract and upper aerodigestive tract and are a causative factor of HNSCC, OPSCC, and oral cancer. Mucosal types can be subdivided into low-risk and high-risk types based on oncogenic potential. The most relevant low-risk types are HPV 6 and 11, and HPV 40, 42, 43, 44, 54, 61, 70, and 72 which can be observed in benign genital mucosal lesions. HPV 31, 33, 35, 52, 58, and 67 are known to be moderate to high-risk, and among the high-risk types, HPV 16 and 18 are most common, and type 16 can be found in various cancers such as cervical cancer, OPSCC, and penile carcinoma[15,16,17].

Host genetic risk factors may also predispose an individual to persistent HPV infection [18]. Also, defective immune response due to genetic variations like inflammasome genetics has proven to be associated with cervical cancer [19]. Several studies showed that EUR-350 T and EUR-350 G, which are HPV16 E6 variants, could influence viral persistence [20]. The integration of HR mucosal HPV genome into host chromosomes in anogenital and oropharyngeal cancers is considered an important driver of carcinogenesis.The gold standard for the diagnosis of HPV in oral cavity is PCR to demonstrate viral genome. The early proteins in HPV genome, E6 and E7 have critical functions in malignant transformation of squamous cells [21]. E6 inactivates TP53 by ubiquitin dependent degradation & E7 manipulates and degrades the retinoblastoma tumour suppressor protein (Rb), resulting in the activation of the transcription factor E2F, which enhances the expression of the cyclin dependent kinase inhibitor 2A (CDKN2A; p16) [22]. So, the expression of p16 protein in the tissue is also considered as a surrogate marker for the diagnosis of HPV [3].

Human papillomavirus (HPV) is a cause of a subset of HNSCC, particularly oropharyngeal squamous cell carcinoma (OPSCC).Head and neck squamous cell carcinoma (HNSCC) is the most common type of HNC(head & Neck Cancer); its incidence is increasing and predicted to rise to 1.08 million new cases per year by 2030[23]. Studies done by Alemany, Laia *et al*[24] shown that HPV-attributable fractions (HPV-AFs) in cases of OPSCC are heterogeneous by geographic region, with HPV-AFs ranging from less than 10% in some world regions to more than 80% in the United States (US) and Northern Europe[25].Differences by sex have also been observed, with higher HPV-AFs in men compared to women, depending on the region. These differences may reflect temporal, geographical, and sociodemographic changes in smoking and sexual behaviour. A systematic review by Mena et al. (2019) reported a global oral HPV prevalence of 4.9% [25].HR-HPV prevalence was 2- to 4-fold higher among men compared with women in each country. Women have a more robust immune response to HPV compared with men [26]. In the present study also, we found a male predilection of (75%).

In a study conducted by Fonseca et al in 2023 the pooled prevalence of HPV-positivity was 10% in the oral cavity and 42% in oropharynx. HPV16 is the genotype most frequent with 69% in OSCC and 89% in OPSCC, being the tonsils the intraoral location

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more affected by HPV [27]. The HPV+ OSCC proportion variated widely and ranged from 0% to 37% [28]. In the present study more cases were seen in tongue followed by buccal mucosa and palate , which is consistent with the findings in the study conducted by Tokuzen *et a*l in 2021[28] & Katirachi *et al* in 2023[29]. Tumours in the tongue were the predominant sub location for HPV in the oral cavity & less common in floor of mouth.

In OSCC without habits, HPV might play a greater relative role, but data are limited and many of those HPV positives show immuno-profiles inconsistent with the classical HPV E6/E7 driven pathway. A study done by More et al 2020[30] compared OSCC with known risk factors Vs OSCC without known risk factors in India. They found HPV16 in 1 of 15 in the "habits" group and 3 of 15 in the "no-known-risk-factor" group. In the present study, mean value of P16 expression was found to be 1.4 in non-habituate persons compared to 0.95 in habituate person. p16 over expression can also be seen in a minority of HPV-Negative OSCC. The possible mechanisms for this over expression are due to epigenetic or genetic alterations of CDKN2A, senescence-associated p16 expression and through non viral mechanisms.

While p16 over expression is a well-established surrogate marker of high-risk HPV-driven disease in oropharyngeal carcinoma, its role in the oral cavity (OSCC) is much more ambiguous. p16 positivity in HPV-negative OSCC is not uncommon, but such cases should not be assumed to neither reflect HPVmediated oncogenesis nor be given a favourable prognostic interpretation. The contribution of HPV prevalence in head and neck squamous cell carcinoma and in particular that of HPV16 in the oropharynx shows the potential benefit of prophylactic vaccines. Incorporating p16 analysis into diagnostics can improve identification and enable personalized treatment approaches.

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