ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Expression Of E6 Oncoprotein Of HPV 16/18 In Oral Epithelial Hyperplasia, Dysplasia, And Oral Squamous Cell Carcinoma.

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ABSTRACT

Most oral squamous cell carcinoma (OSCC) cases have known associated risk factors including tobacco, alcohol, areca nut, etc. In addition to this, there has been an ongoing debate as to the etiology of oral cancer in patients without any known risk factors. In such cases, a microbial etiology has been hypothesized. Human papillomavirus (HPV) a proven risk factor for cervical and oropharyngeal cancer has been closely associated with oral cancer, although conclusive evidence for causal inference is not established. Significant epidemiological evidence exists associating HPV and oral cancer, especially the high-risk types 16 and 18. This study aims to evaluate the expression of E6 oncoprotein of human papillomavirus 16/18 in oral epithelial hyperplasia, oral epithelial dysplasia & oral squamous cell carcinoma and to compare the expression of E6 oncoprotein of human papillomavirus 16/18 among the different study groups. A total of 45 samples that included 15 cases of oral epithelial hyperplasia, 15 cases of oral epithelial dysplasia, and 15 cases of oral squamous cell carcinoma were included in the study. All the parameters were tabulated and assessed for statistical significance using the statistical package for Social Science (SPSS) software version 20. Immunopositivity of the different grades of squamous cell carcinoma was statistically analyzed using Fisher's exact test. The p-value obtained was 1.000 showing that there was no statistically significant difference in immunopositivity among different grades of oral squamous cell carcinoma. The intensity of staining for E6 oncoprotein HPV 16/18 was assessed subjectively as mild, moderate, and intense. On assessing the koilocytosis positivity within different grades of oral squamous cell carcinoma we found that one out of 6 cases of well differentiated squamous cell carcinoma and three out of 8 cases of moderately differentiated squamous cell carcinoma showed positivity for koilocytosis. The only one case of poorly differentiated squamous cell carcinoma that was examined for positivity of koilocytosis also showed to be positive. There was a statistically significant difference in immunopositivity for E6 oncoprotein HPV 16/18 among the three different groups and a moderately strong association between the study groups and immunopositivity for E6 oncoprotein HPV 16/18.

Keywords: Human papillomavirus (HPV), oral squamous cell carcinoma (OSCC), oral epithelial dysplasia, risk factors, tobacco.

https://doi.org/10.33887/rjpbcs/2023.14.6.43

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ISSN: 0975-8585

INTRODUCTION

Oral lesions have different etiologies, although many of them can be largely attributed to environmental exposures. Tobacco use, chewing areca nuts, and alcohol consumption are well-established risk factors for various lesions of the oral cavity [1]. Infectious agents also play an important role in the etiology of oral lesions. Among them, human papillomaviruses (HPV) seem to be associated with a subset of oral benign proliferative and malignant lesions, and the head and neck carcinoma, notably carcinoma of the oropharynx, tonsils and tongue. HPV is a small, epitheliotropic, non-enveloped DNA virus [2]. The HPV genome consists of 7200 to 8000 base pairs of closed-circular double-stranded DNA, containing up to10 open reading frames. HPV infection is the most common of all sexually transmitted diseases. Oral HPV infection can be acquired by oral-genital contact, mouth-to-mouth contact, or possibly by autoinoculation, and in infants by mother-to-child transmission [3]. Human papillomavirus (HPV) causes a wide spectrum of diseases affecting the cutaneous and mucosal areas of the body, ranging from benign common warts to invasive carcinoma. HPV infections have been reported in several body sites, including the anogenital tract, urethra, skin, larynx, tracheobronchial mucosa, nasal cavity, paranasal sinus, and oral cavity. Oral HPV infection may be associated with different diseases of the oral cavity [4]. To date, more than 200 different HPV types, ranging from HPV-1 to HPV-210 have been officially recognized by the International HPV Reference Center [5]. Four of the previously recognized HPV types (HPV-46, HPV-55, HPV-64, and HPV-79) were recently re-classified as subtypes. HPV types infecting the mucosa are further classified into high and low-risk groups based on the type of lesions they cause. Low-risk type HPVs like HPV-6 and HPV-11 cause benign warts. High-risk HPVs, such as HPV-16 and HPV-18, cause premalignant squamous intraepithelial neoplasia that can progress to cancer. Generally, oral epithelium undergoes a sequence of histopathological changes like hyperplasia and dysplasia before the development of invasive carcinoma [6]. The specific role of human papillomaviruses (HPV) in the development of premalignant lesions and oral squamous cell carcinoma (OSCC) continues to be a much-debated topic [7]. Recently, the common term "oral potentially malignant disorders" (OPMD) has been suggested to include both oral precancerous lesions (e.g. leukoplakia, erythroplakia, oral proliferative verrucous leukoplakia) and oral precancerous conditions (e.g. lichen planus, submucous fibrosis). All oral mucosal lesions that carry a risk of malignant transformation are included under this term [8]. Leukoplakia is the most common potentially premalignant lesion in the oral cavity. Tobacco and areca nut use, either alone or in combination, are the most common risk factors for oral leukoplakia, although some are idiopathic. Leukoplakia may unpredictably regress, remain stable, or progress to carcinoma [9]. There is a greater risk of carcinomatous transformation of idiopathic leukoplakia, non-homogenous leukoplakia, leukoplakia affecting the high-risk sites, and leukoplakia in which the keratinocytes carry cytogenic alterations associated with carcinomatous transformation Although there appears to be some link between HPV and oral leukoplakia, there is little evidence to support a causal relationship either between HPV infection and oral leukoplakia or between HPV infected keratinocytes and their malignant transformation [10]. Oral lichen planus (OLP) is a chronic autoimmune disorder of unknown etiology in which predominantly T-lymphocytes accumulate beneath the epithelium and increase the rate of differentiation of stratified squamous epithelium, resulting in either epithelial thickening or atrophy with or without ulceration [11]. HPV-associated carcinogenesis is mediated by expression of the viral E6 and E7 oncoprotein, which cause dysregulation of the cell cycle by inactivating p53 and RB gene respectively. Hence this study was designed to evaluate the presence of HPV in oral epithelial hyperplasia, dysplasia, and oral squamous cell carcinoma using the E6 oncoprotein of HPV 16/18 immunohistochemical marker that might help in better understanding of the role played by virus in oncogenic process from its evolution stage [12]. Investigation into the role of HPV could be rewarding in planning long term strategies for prevention, diagnosis, and possible treatment options for these (leukoplakia, oral submucous fibrosis, lichen planus, and oral squamous cell carcinoma) conditions [13,14].

MATERIALS AND METHODS

This study was conducted in the Department Of Pathology, Karpagam Faculty of Medical Sciences & Research, Coimbatore, Tamil Nadu, India from October 2021 to October 2023. A total of 45 samples that included 15 cases of oral epithelial hyperplasia, 15 cases of oral epithelial dysplasia, and 15 cases of oral squamous cell carcinoma were included in the study.

Inclusion Criteria

Tissue blocks of histopathologically diagnosed cases of oral epithelial hyperplasia, oral epithelial

ISSN: 0975-8585

dysplasia, and oral squamous cell carcinoma were included.

Exclusion Criteria

Tissue blocks of the above-mentioned cases, with inadequate tissue were excluded. Histologically diagnosed cases of cervical carcinoma were included as positive controls. For each batch of immunohistochemical staining, one positive control slide of cervical cancer and one negative control slide without incubating with primary antibody were also stained concurrently. It was ensured that the positive control slide showed specific positive immunoreactivity to Anti E6 HPV 16/18 antibody used in every batch. In the event of positive control showing negative immunoreactivity, the entire batch was rejected.

Statistical Methods

All the parameters were tabulated and assessed for statistical significance using the Statistical Package for Social Science (SPSS) software version 20. The difference in the expression of E6 oncoprotein HPV 16/18 and the presence of koilocytosis in oral epithelial hyperplasia, dysplasia, and oral squamous cell carcinoma were statistically analyzed using Fisher's exact test to compare individual groups against each other, followed by Cramer's V test to assess the strength of association between the parameters.

Table 1: Distribution Of Cases Among The Three Study Groups

Groups	Number Of Cases
Oral epithelial hyperplasia	15
Oral epithelial dysplasia	15 (Mild - 4; Moderate - 10; Severe - 1)
Oral Squamous cell carcinoma	15 (Well differentiated - 6; Moderately differentiated - 8; Poorly differentiated - 1)

Table 1: A total number of 45 cases, comprising 15 cases of oral epithelial hyperplasia [Group I], 15 cases of oral epithelial dysplasia [Group II], and 15 cases of oral squamous cell carcinoma [Group III] were included in the study. Percentage of positivity of E6 HPV 16/18 in the different study groups Immunohistochemical expression of E6 oncoprotein HPV 16/18 was assessed in allthree groups. Group I: In this group, none of the 15 cases of oral epithelial hyperplasia showed immunopositivity for E6 oncoprotein HPV 16/18, and the total percentage of positivity was 0%. Group II: In this group, none of the 15 cases of oral epithelial dysplasia, irrespective of their grade, showed immunopositivity for E6 oncoprotein HPV 16/18, and the total percentage of positivity was 0%.Group III: Four out of 15 cases of oral squamous cell carcinoma showed immunopositivity for E6 oncoprotein HPV 16/18 and the percentage of positivity was 27%.

Table 2: Cross Tabulation Of Positivity For E6 HPV 16/18 With The Different Study Groups

			IHC Positivity		Total
			No	Yes	
Group	OEH	Count	15	0	15
		% within Group	100.0%	0.0%	100.0%
		% within IHC positivity	36.6%	0.0%	33.3%
	OED	Count	15	0	15
		% within Group	100.0%	0.0%	100.0%
		% within IHC positivity	36.6%	0.0%	33.3%
	OSCC	Count	11	4	15
		% within Group	73.3%	26.7%	100.0%
		% within IHC positivity	26.8%	100.0%	33.3%
		% of Total	24.4%	8.9%	33.3%
			Value	Exact Sig. (2-sided)	
Fisher's Exact Test			6.419	.027	
Symmetric Measures					
			Value	Approx. Sig.	Exact Sig.
Cramer's V			.442	.012	.027

ISSN: 0975-8585

Table 2: Immunopositivity of E6 oncoprotein HPV16/18 in different groups was compared. Results obtained were analyzed using Fisher's exact test The p-value obtained was 0.027, showing that there is a statistically significant difference in immunopositivity among different groups. Using Cramer's V value (0.442) to estimate the strength of association. We could determine that there was a moderately strong association between the study groups and immunopositivity of E6 oncoprotein HPV 16/18.

Table 3: Cross-Tabulation Of Positivity For E6 HPV 16/18 With The Different GradesOf Oral Squamous Cell Carcinoma

			IHC Positivity		Total
			No	Yes	
Group	WDSCC	Count	4	2	6
		% within Group	66.7%	33.3%	100.0%
		% within IHC positivity	36.4%	50.0%	40.0%
	MDSCC	Count	6	2	8
		% within Group	75.0%	25.0%	100.0%
		% within IHC positivity	54.5%	50.0%	53.3%
	PDSCC	Count	1	0	1
		% within Group	100.0%	0.0%	100.0%
		% within IHC positivity	9.1%	0.0%	6.7%
			Value	Exact Sig. (2-sided)	
Fisher's Exact Test			.782	1.000	
Symmetric Measures					
			Value	Approx. Sig.	Exact Sig.
Cramer's V			.185	.774	1.000

Table 3: Immunopositivity of the different grades of squamous cell carcinoma was statistically analyzed using Fisher's exact test. The p-value obtained was 1.000 showing that there was no statistically significant difference in immunopositivity among different grades of oral squamous cell carcinoma. Using Cramer's V value (0.185), we determined that there was a very weak association between the immunopositivity of E6 oncoprotein HPV 16/18 and the grading of oral squamous cell carcinoma.

Table 4: Overall comparison of intensity of E6 HPV 16/18 expression in different grades of oral squamous cell carcinoma group

Group	Total no ofcases	Mild (%)	Moderate(%)	Severe(%)	No expression(%)
Oral squamous cell carcinoma	15	4 (27%)	0 (0%)	0 (0%)	11 (73%)

Table 4: The intensity of staining for E6 oncoprotein HPV 16/18 was assessed subjectively as mild, moderate, and intense. We found that all four cases that showed immunopositivity for E6 oncoprotein took up only mild intensity of the stain irrespective of the group or subgroup they belonged to. Quantification of immunopositivity was also done by calculating the average number of cells per high-power field that were positive for E6 stain. We found that: One case of well-differentiated squamous cell carcinoma showed 4% positive nuclei per high-power field. One case of well-differentiated squamous cell carcinoma showed 23% positive nuclei per high-power field. One case of moderately differentiated squamous cell carcinoma showed 4% positive nuclei per high-power field. One case of moderately differentiated squamous cell carcinoma showed 18%positive nuclei per high-power field.

Table 5: The Total number of koilocytes was evaluated in all three groups. An average number of koilocytes per high-power field was assessed for each case. The presence of one or greater than one number of koilocytes per high-power field was considered to be positive for koilocytosis. GROUP I: In this group, five out of 15 cases of oral epithelial hyperplasia showed positivity for the presence of koilocytes, and the total percentage of positivity for koilocytosis was 33.33%.GROUP II: In this group, out of 15 cases of oral epithelial dysplasia, two cases showed positivity for the presence of koilocytes and the total percentage of positivity was 13.33%.GROUP III: In this group, five out of 15 cases of oral squamous cell carcinoma, showed positivity for the presence of koilocytes and the total percentage of positivity was 33.33%.We compared the presence of koilocytosis among the cases in all three study groups. The result

ISSN: 0975-8585

obtained was statistically analyzed using Fisher's exact test. The p-value obtained was 0.414. suggesting that there was no significant difference in the positivity of koilocytosis between the different groups. Using Cramer's V value (0.213), we found a weak association between the positivity of koilocytosis and different groups.

Table 5: Percentage of positivity of koilocytosis in the three groups

			Koilocytosis		Total
			No	Yes	
Group	OEH	Count	10	5	15
		% within Group	66.7%	33.3%	100.0%
		% within Koilocytosis	30.3%	41.7%	33.3%
	OED	Count	13	2	15
		% within Group	86.7%	13.3%	100.0%
		% within Koilocytosis	39.4%	16.7%	33.3%
	OSCC	Count	10	5	15
		% within Group	66.7%	33.3%	100.0%
		% within Koilocytosis	30.3%	41.7%	33.3%
			Value	Exact Sig. (2-sided)	
Fisher's Exact Test			2.098	.414	
Symmetric Measures					
			Value	Approx. Sig.	Exact Sig
Cramer's V			.213	.360	.522

Table 6: Crosstabulation Of Koilocytosis With The Grades Of Oral Squamous Cell Carcinoma

			Koilocytosis		Total
			No	Yes	
Group	WDSCC	Count	5	1	6
		% within Group	83.3%	16.7%	100.0%
		% within Koilocytosis	50.0%	20.0%	40.0%
	MDSCC	Count	5	3	8
		% within Group	62.5%	37.5%	100.0%
		% within Koilocytosis	50.0%	60.0%	53.3%
	PDSCC	Count	0	1	1
		% within Group	0.0%	100.0%	100.0%
		% within Koilocytosis	0.0%	20.0%	6.7%
			Value	Exact Sig. (2-sided)	
Fisher's Exact Test			2.550	.254	
Symmetric Measures					
			Value	Approx. Sig.	Exact Sig.
Cramer's V			.433	.245	.254

Table 6 On assessing the koilocytosis positivity within different grades of oral squamous cell carcinoma we found that one out of 6 cases of well-differentiated squamous cell carcinoma and three out of 8 cases of moderately differentiated squamous cell carcinoma showed positivity for koilocytosis. The only one case of poorly differentiated squamous cell carcinoma that was examined for positivity of koilocytosis also showed to be positive. We compared the positivity of koilocytosis among the different grades of oral squamous cell carcinoma. Using Fisher's exact test, the p-value obtained was 0.254. This showed that there was no significant difference in the prevalence of koilocytosis between the different grades of oral squamous cell carcinoma. Using Cramer's V value (0.433), we determined that the strength of association between the positivity of koilocytosis and different grades of oral squamous cell carcinoma was moderately strong.

DISCUSSION

Most oral squamous cell carcinoma (OSCC) cases have known associated risk factors including tobacco, alcohol, areca nut, etc. In addition to this, there has been an ongoing debate as to the etiology of

oral cancer in patients without any known risk factors. In such cases, a microbial etiology has been hypothesized [15]. Human papillomavirus (HPV) a proven risk factor for cervical and oropharyngeal cancer has been closely associated with oral cancer, although conclusive evidence for causal inference is not established [16]. The presence of HPV DNA in oral cancer tissue and that of high-risk HPV viruses and altered healthy oral epithelial cells support the idea that HPV has a role as an etiological agent in oral cancer. In light of this, the current investigation was done to check for the presence of HPV types 16 and 18 in oral epithelial dysplasia [17]. A significant change in oral potentially malignant disorders (OPMD) and OSCC incidence was because of a decrease in the number of cases associated with tobacco, while new cases were due to HPV [18]. The etiopathogenesis of squamous cell carcinoma is important as HPV-associated OSCC and OPMD have higher curing rates than those associated with tobacco and alcohol risk factors. Unfortunately, approximately 2/3 of lesions were identified at an advanced stage, which affected treatment options, requiring more complex therapy, and increasing the morbidity of treatment and cost of care. It is expected that management of OPMD and early-stage squamous cell carcinoma leads to a better prognosis [19]. Although most OSCC cases are expected to be preceded by OPMD, it is not known whether OPMD arises from potentially detectable precursor lesions. [20]. Although early detection of OPMD and OSCC is a desirable goal, evidence supporting the screening is limited, because the progression of oral lesions to cancer cannot be predicted. Dysplasia or even early cancer may be resolved without treatment, which complicates diagnosis and treatment decisions [21]. A focus on high-risk populations where prevalence is greater may increase the potential value of screening. The complications regarding screening for low-prevalence diseases lead to challenges in detection an increased risk of false-positive and false-negative outcomes and higher costs. These challenges continue to challenge oral cancer detection. The current best evidence is limited to high-risk populations, such as those with prior upper aerodigestive tract cancer, exposure to heavy tobacco and alcohol use, exposure to HPV, and immunosuppression [22]. The prevalence of HPV in oral epithelial dysplasia and its association with advancing a risk prediction model for the malignant progression of oral epithelial dysplasia can provide further insight into the risk of stratification of oral potentially malignant disorders [23]. To validate the prevalence of HPV in oral epithelial dysplasia and its association with developing a risk prediction model for the malignant progression of oral epithelial dysplasia, this study aimed to determine whether the repeated measurements of clinical features of OPMDs (lesion presence, size, appearance, color, texture, and histopathology) predict malignant progression [24].

CONCLUSION

E6 oncoprotein expression is noticed in a few cases of oral squamous cell carcinoma. However, they are not expressed in cases of oral epithelial hyperplasia and oral epithelial dysplasia, suggesting that their role may be limited to a few but not all cases of oral squamous cell carcinoma. Since the prevalence of koilocytosis was noticed in more cases in all the groups, a transient viral infection may be noticed in these oral lesions as a co- infection. However, whether they play a direct role in carcinogenesis in the oral cavity needs further assessment. It is also possible that the low prevalence of HPV in our study could be due to the component of the virus (i.e. E6 oncoprotein) chosen to be examined and its role in oral potentiallymalignant disorders and oral squamous cell carcinoma needs to be established. Finally, it is possible that the immunohistochemistry method is not sufficiently sensitive toidentify HPV components in oral lesions. Studies in the same population using advanced molecular methods like in situ hybridization or polymerase chain reaction may be more beneficial to ascertain the role of HPV 16/18 in oral premalignant and malignant lesions.

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