



Original Article

Comparison of P16 with Human Papillomavirus with Oral Squamous Cell Carcinoma and Oral Premalignant Lesion

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ABSTRACT

Worldwide Oral carcinomas (OSCC) are a considerable problem. It is crucial to know the molecular incidences of molecular carcinogenesis, as it has a significant association with tobacco in Pakistani population. Objective: To screen out the savage high risk of papilloma virus 16/18 and p16 in oral premalignant lesion (OPL) and oral squamous cell carcinoma. Moreover, the p16 correlation between HPV and OPL and OSCC was also analyzed. Methods: A total of 150 samples from the oral cavity were taken from the Hayatabad Medical complex (50 samples of OPL and 100 samples of OSCC). In this study immunohistochemistry for p16 in OPL and OSCC was performed, and polymerase chain reaction was done for HPV. SPSS 21 was used for data analysis. The relationship between HPV and p16 with different variables was determined using Chi-square and Fisher exact tests. Results: For p16, 14% of 50 OPLs were found positive and 86% were negative. Moreover, while screening for the OSCC, the 18 percent individuals were found positive for OSCC, while the remaining (82%) screened out negative. HPV was diagnosed in 6% of 50 OPL. The co-occurrence of HPV with p16 was found in all the 15 (100%) individuals with a p value of 0.001. However, three of the 18 cases with p16 expression did not have HPV infection. Conclusions: p16 may be suggested as a unique marker for HPV in OSCC. Furthermore, a chemical carcinogen like cigarette is thought to be one of the main risk factors for p16 and HPV infection, as well as other things.

INTRODUCTION

Oral cancer is the eighth most prevalent cause of cancer-related mortality in the world [1]. GLOBOCAN estimates show that cancer of the mouth is one of the main causes of death in developing countries. Head and neck carcinoma is the sixth widest prevalent malignant tumor worldwide and is the utmost serious public health issue. Oral malignancies come in a variety of forms, but squamous cell carcinomas account for over 90% of all cases [2]. OSCC is estimated to

be responsible for approximately 220000 new cases per year (5 percent of all malignancies) worldwide [3]. The human papillomavirus (HPV) is now known to be an occurrence factor for (OSCC), especially in the throat region. Variations in HPV connection with the oral squamous cell carcinomas and oral premalignant lesions vary widely in various studies throughout the globe, ranging from 0% to 100% [4]. On chromosome 9p21, the p16 protein

is a tumor suppressor or gene. In the early phases of carcinogenesis, p16 expression is lost [3,5]. Furthermore, individuals with OSCC who have been exposed to traditional risk factors have lost p16 due to processes [6]. According to the literature, P16 overexpression was found in tumors with HPV infections that were still going on. The p16 transcription-initiation is usually inhibited by pRB, but when HPV infection occurs, an E7 protein of HPV induces pRB to become activated, resulting in p16 overexpression [7]. The p16 immunoreactivity has a strong link with HPV occurrence because p16 overexpression has a chance to become a surrogate diagnostic for HPV detection. However, as compared to primary OSCC, their link seems to be stronger in HNSCC [8]. According to studies, p16 overexpression may be caused by routes other than the HPV E7 protein [9]. As a result, the effect of p16 overexpression as a unique marker in HPV-infected oral squamous cell carcinomas must be investigated. The goal of this research was to look at the overexpression of p16 in OPL and OSC carcinomas, find HR-HPV16/18 in oral squamous cell carcinomas and oral premalignant lesion, and look at the relationship between HPV16/18 and p16.

METHODS

After receiving ethical permission from the IRB, the research was carried out at Khyber College of Dentistry. A total of 150 samples from the oral cavity were taken from the Hayatabad Medical complex (100 of OSCC and 50 cases of OPL). The patients' clinic pathological data were recorded. Addictions like smoking, snuffing and tobacco chewing were assessed as risk factors for OPL and OSCC in great detail. For histological and molecular comparison, 25 normal and 25 affected patients were included in this study. The routine grossing, biopsy, and hematoxylin and eosin (HandE) staining were carried out. Barnes classified OPL as moderate, mild, or severe dysplasia on histopathological examination. The guidelines of the WHO for OSCC were categorized as; Grade I (well-differentiated), grade II (moderately differentiated), and grade III (extremely differentiated) according to World Health Organization (WHO) guidelines (poorly differentiated). Clinical staging of OSCC patients was documented according to the AJC Staging (stage I-IV) [9]. All patients were treated with a standard immunohistochemical process to look into the countenance of p16 in OSCC and OPL. The sections are deparaffinized, rehydrated, and antigen retrieval was completed. Mouse primary monoclonal human antibodies against p16 (5A8A, Abbie-tec) are incubated with exposed sections for 1 hour in the moisturizing chamber. Abbiotec made a secondary antibody (HRP-conjugated anti-rabbit antibody) that could be used as an amplifier. Solution B was used as the polymer. Staining was controlled for all

readouts by utilizing common p16-manipulating normal mucosal specimen and instances of hyperplasia. The immune-stained slides were examined by two experienced pathologists. All the samples were evaluated by diffuse nuclear and cytoplasmic Stains by p16. A semi-quantitative method was used to figure out how much p16 stain. This meant multiplying the intensity of the stain and the percentage of cells that were affected by the stain. The proportion of stained cells and level of intensity was assessed as in Table.1 below

No staining	0	Weak	1
0-10 percent-stained cells	1	Moderate	2
11-50 percent-stained	2	Strong	3
51-80 percent-stained cells	3		
81-100 percent-stained cells	4		

Table 1: The proportion of stained cells and level of intensity. Multiplying the two variables yielded a final score of 0-12. The case is deliberated positive if the score was found to be 4 or above. Whole specimens were gathered and sliced into 5-micron slices. DNA was extracted from tissue sections using the DNA tissue kit (Qiagen, Valencia, CA) (Invitrogen Life Technologies, Carlsbad, USA). The HPV genotype was determined using 12.5 l of isolated DNA and Real-time PCR. A real-time PCR kit (HPV16/18RealTMQuant, Sacace Biotechnologies, Italy) was used to quantitatively genotype human papilloma virus-16 and human papilloma virus-18 in isolated DNA (SmartCyclerII, Cepheid, USA). SPSS version 21 was used to record gender, age, type, grade, HPV expression, stage, and p16. The frequency of the above variables was determined using descriptive statistics. To find out how p16 expression and HPV are linked, researchers looked at a lot of different things about people.

RESULTS

Among all the 50 OPL cases, included in this study have average age of 40. Thirty-five (70%) of them were male, whilst 15 (30%) were females. The bulk of oral premalignant lesion patients (92%) have become accustomed to risk factors. Check was the most common OPL linguistics site, accounting for 21 (42 percent). Clinically, leukoplakia was the most frequently diagnosed OPL (40%). There were 25 patients who were found with mild grade dysplasia, while the incident rate of moderate grade dysplasia is in sixteen individuals. Furthermore, nine individuals were found with severe grade dysplasia. Out of the total 100 OSCC cases, the patients' average age was 48 years old. The research comprised of 74 males and 26 females. In 91 percent of cases, patients were exposed to risk factors. The most

prevalent location of OSCC lesion was the cheek (50 percent). Histologically, (48%) patients had grade I tumors, (37%) had grade II tumors and 15 had grade III tumors. Six cases of stage I cancer, 20% cases of stage II cancer, 35% cases of stage III cancer, and 39% cases of stage IV cancer were found. Out of 50 cases of oral premalignant lesion, 43 (86 percent) demonstrated no expression of p16, whereas 7 (14 percent) indicated p16 overexpression. There was no significant association between p16 loss and overexpression. Lack of p16 expression was seen in 82% of the 100 percent instances of OSCC, whilst over-activation of p16 was observed in 18 (18%) of the patients. There was a very significant association between p16 negative instances and tumor differentiation, with the majority of cases being found in grade I and grade II malignancies (p = 0.000). The statistically significant association found between negative cases and risk factors (p<0.05) There was no evidence of a link between demographic factors of the individuals as well as tumor size or cancer stage and the loss of p16 expression (Table 2). In addition, HPV was found to be positive in 15 of the 100 cases with OSCC. HPV was primarily positive in individuals who smoked and chewed betel quid (p = 0.029), indicating a substantial link between HPV and risk factors. There was also a significant link between HPV and tumor grade (p = 0.001) though, with another variable no significant correlation was observed (Figure 2)(Table 3). Three (6%) of the 50 OPL patients were HPV positive. Other characteristics such as gender, age, location, clinical and histological grade and risk factors of the tumor had no significant relationship with HPV positivity. According to OPL, HPV DNA presence in dysplastic epithelial cells was closely associated with p16 overexpression in 3 of 3 (100%) cases. In real-time PCR, however, four out of seven p16-positive patients did not reveal HPV DNA (Table 3). HPV co-expression with p16 was in all 15 (100%) cases of HPV positive OSCC cases (Table 4).

Parameters	P16		Total	Chi-Squared Test
	Negative	Positive		
Age Groups				
21-30yrs	6(66.7%)	3(33.3%)	9	0.3684
31-40yrs	2(83.3%)	4(16.7%)	24	
41-50yrs	3(88.2%)	4(11.8%)	34	
51-60yrs	18(81.8%)	4(18.2%)	22	
61-70yrs	6(75%)	2(25%)	8	
71-80yrs	2(66.7%)	1(33.3%)	3	
Genders				
Males	58(78.4%)	16(21.6%)	74	0.112
Females	24(92.3%)	2(7.7%)	26	

RiskFactors				
NoHabits	8(88.9%)	1(11.1%)	9	0.002*
Tobacco Smoking	9(56.3%)	7(43.8%)	16	
BQ/Tobaccochewing	44(95.7%)	2(4.3%)	46	
Smoking with BQ/Tobaccochew	21(72.4%)	8(27.6%)	29	
Locations				
Check (head and neck region)	40(80%)	10(20%)	50	0.657
Tongue (head and neck region)	21(87.5%)	3(12.5%)	24	
Lip (head and neck region)	5(71.4%)	2(28.6%)	7	
Alveolus(Head and Neck Region)	8(88.9%)	1(11.1%)	9	
Palate(Head and NeckRegion)	4(66.7%)	2(33.3%)	6	
Floor(Head and Neck Region)	4(100%)	0(0%)	4	
Stage				
Stage:I	4(66.7%)	2(33.3%)	6	0.526
Stage:II	17(85%)	3(15%)	20	
Stage:III	31(88.6%)	4(11.4%)	35	

Table 2: Association between p16 and oral squamous cell carcinomas

Variables	HPV		Total	Chi- squared test
	Negative	Positive		
Age Groups				
21-30yrs	7(66.7%)	3(33.3%)	9	0.442-
31-40yrs	20(87.5%)	4(12.5%)	24	
41-50yrs	32(88.2%)	4(11.8%)	34	
51-60yrs	21(90.9%)	2(9.1%)	22	
61-70yrs	6(75%)	2(25%)	8	
71-80yrs	3(66.7%)	2(33.3%)	3	
Genders				
Males	60(82.4%)	13(17.6%)	74	0.342
Females	25(92.3%)	2(7.7%)	26	
Risk Factors				
No Habits	9(88.9%)	2(11.1%)	9	0.029*
Tobacco Smoking	12(75%)	4(25%)	16	
BQ/Tobaccochewing	45(95.7%)	1(4.3%)	46	
Smoking with BQ/Tobacco Chewing	22(72.4%)	7(27.6%)	29	
Location				
Cheek(HN-HPV)	49(84%)	10(16%)	50	0.510-
Tongue(HN-HPV)	22(91.7%)	2(8.3%)	24	
Lip(HN-HPV)	5(71.4%)	2(28.6%)	7	
Alveolus(HN-HPV)	8(88.9%)	1(11.1%)	9	
Palate(HN-HPV)	4(100%)	0(0%)	4	
Floor(HN-HPV)	4(66.7%)	2(33.3%)	6	
Stage				
Cancer Stage:I	4(66.7%)	2(33.3%)	6	
Cancer Stage:II	18(90%)	2(10%)	20	

Table 3: Association between human papilloma virus and oral squamous cell carcinomas

Oral Premalignant Lesions				P- value	Oral Squamous Cell Carcinomas			P- value
P16	HPV +ve	HPV -ve	Total		HPV +ve	HPV -ve	Total	
+ve	4	5	9	* <0.001	15	3	18	* <0.001
-ve	0	43	43		0	82	82	

Table 4: Association between p16 and human papilloma virus and in oral premalignant lesion and oral squamous cell carcinomas

DISCUSSION

OSCC was always thought to be a disease of the elderly, but in recent decades, it has become more frequent in younger age groups, as shown by our research [10]. This transition to ten years ago is most likely due to early exposure to chemical carcinogens like cigarettes and betel quid. The current research found that 91 percent of OSCC were routinely exposed to chemical carcinogens, with a male preponderance, and that the majority were engaged in both habits of smoking and tobacco chewing, which supports the twofold frequency of men afflicted by OSCC [6]. A half or more of the incidences (59%) were found in the cheeks, which is consistent with the bulk of localized investigations [11]. Studies in the West have identified the tongue as the most common site of OSCC [12]. This is most likely elaborated by the addiction of placing snuff or tobacco, or associated quids at a specific location. Several types of research in recent decades have highlighted the molecular changes that occur as a result of different grades of dysplasia, leading to changes in the phenotype of invasive malignancy. The roles of HPV and p-16 in oral squamous cell carcinomas and oral premalignant lesions are currently being studied. Other studies have found a similar trend in HPV incidences (15 percent = oral squamous cell carcinomas and 6 percent = oral premalignant lesions) in our study [12]. Interestingly, a greater frequency of HPV has been seen in head and neck squamous cell cancer (HNSCC) in various investigations [13]. This gap may be explained by the fact that high-risk HPV-related malignancies were most typically seen in the buccal cavity as it is included in HN-HPV classifications but not in the WHO classification of the oral cavity. We confirmed the findings of prior research that high risk-16 is more commonly the cause of oral cell squamous cell carcinomas HR-HPV-18 [3,14]. Despite the fact that research suggests that HPV-independent occurrence factor for oral squamous cell carcinomas. It was also found that tobacco-related carcinogens cause genetic changes that may make HPV-positive tumors less susceptible to treatment. Our research found a substantial link between human HPV cases and chemo-risk factors, notably in those who chew tobacco, indicating that, in addition to snuffing and smoking, which is widely used in our area, has a major link with HPV infection. There is a confined link between HPV-infected individuals and high-grade malignancies, that is comparable to Patilet's results, and this might be related to viral excess and aggressive tumor behavior [15]. Changes in the p16 tumor suppressor gene are very usual and the only molecular events in oral carcinogenesis. Mostly, high risk HPV is unconcerned with oral cancers, p16 inhibition via gene silencing leads to its mall expression. Similarly, high rates of p16 inactivation, 86 percent in OPL and 82 percent in OSCC, were found in our research with a

strong connection to moderate dysplasia and low-grade tumors, which are defined as an early event by previous studies [16]. Likely, 86.6 percent of OSCC patients tested positive for p16 and 87 percent for HPV [17]. However, a few instances with p16 overexpression were found to be HPV non-infected in our investigation. Previous literature evidence that usually p16 occurrence is not affected by the HPV [13]. It is necessary to investigate the utmost plausible details for Rb inhibition induced by sources besides HPV infection. A further suggested that betel quid and cigarettes might play a role in HPV-unrelated cancers with p16 positivity [18]. As a result, not only HPV but also chemical carcinogens may inactivate Rb gene. Alternatively, it's possible that there are undiscovered HPV genotypes that aren't detected by PCR. Another possibility is that cancers have high levels of p16, even if they don't have HPV.

CONCLUSION

The study concluded that HPV was found in a significant proportion of OSCC patients as well as a few OPL cases. The study excluded cancers of the oropharynx and tonsils, which are common sites for HR-HPV-related tumors, and this could explain why the HR-HPV prevalence rates in this series were so low. p16, on the other hand, was expressed in all HPV-positive malignancies in our cohort, indicating that it is a good but not perfect surrogate marker for HR-HPV. The previous literature was found deficient regarding p16 expression, human papilloma viral infection occurrence and risk factors as well as incidences of OPL and OSCC and not were studied in-depth as we have done. We didn't have a lot of time, which was one of the restraints.

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