INCIDENCE OF HUMAN PAPILLOMA VIRUS IN PATIENTS OF ORAL SQUAMOUS CELL CARCINOMA IN A SUBSET OF PAKISTANI POPULATION

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ABSTRACT

Objective: To determine the frequency of immunohistochemical expression of HPV-16 in oral squamous cell carcinoma. Furthermore, to characterize the histological subtypes of squamous cell carcinoma using immuno-histochemistry.

Study Design: Descriptive, cross-sectional study.

Place and Duration of Study: Department of Histopathology, Armed Forces Institute of Pathology, Rawalpindi from Jan 2017 to Jan 2018.

Material and Methods: A total of thirty (n=30) specimen of oral squamous cell carcinoma detected on histopathology were enrolled in the study. Immunohistochemistry analysis was performed, and HPV was detected by applying p16, a surrogate marker of HPV, on formalin fixed and paraffin embedded sections. Mean and standard deviation were calculated for quantitative variable. Frequency and percentages were calculated for qualitative variables. Data was further stratified with respect to gender, age and tumor size.

Results: Immunohistochemistry analysis revealed that there were 83.3% (n=25) samples which were positive for HPV-16. Mean tumor size was 1.9 cm ± 1.4 SD in the study sample (1.8 cm ± 1.1 SD in males and 2.3 cm ± 1.8 SD in females). Tumor size was <2cm in 73.3% (n=22) samples and >2cm in 26.7% (n=8) samples stratification with respect to gender (males and females), age (<60 years and >60 years) and tumor size (<2cm and >2cm) did not reveal any significant difference in HPV positivity.

Conclusion: A significant proportion of specimens of oral squamous cell carcinoma exhibited HPV-16 positivity in our population. No significant difference was noted across gender, age and tumor size.

Keywords: Human papillomavirus, Immunohistochemistry, Oral squamous Cell carcinoma.

INTRODUCTION

Squamous cell carcinoma of the head and neck has long been regarded as a disease entity having a remarkable incidence worldwide and onerous prognosis[1]. Incidence of oral squamous cell carcinoma (OSCC) is approximately 14/100,000 accounting for 16% to 40% of all the malignancies[2]. In Pakistan, the prevalence of oral squamous cell carcinoma has reached 10% of all cancers[3]. There is growing evidence that human papillomavirus (HPV) may act as a cocarcinogen along with tobacco which eventually results in oral squamous cell carcinoma. Human Papillo-maviruses (HPVs) are small, double stranded deoxyribonucleic acid (DNA) viruses belonging to a papillomaviridae family[4]. In 1986, human papillomavirus DNA was first detected in an invasive squamous cell carcinoma by Southern blot hybridization[5]. Over 130 HPV types have been detected and classified as high-risk and low-risk, based on their association with squamous cell carcinoma[6]. HPV transforms infected epithelial cells and causes defects in genes controlling apoptosis, cell-cycle and DNA repair, thereby promoting tumorigenesis[7]. HPV-16 and 18 are the most commonly detected high-risk types in oral squamous cell carcinoma.The protein p16 is a cellular protein involved in cell cycle regulation. In normal cells, p16 protein is expressed in very low levels and is almost undetectable by IHC. Due to the transforming activity of E7 oncogene, p16 is strongly expressed in tumor cells affected by HPV and may be easily

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detected by IHC[8]. Hence, p16 positivity correlates strongly with HPV positivity[9]. Different studies have shown different percentages of expression of p16 in HPV-related oral squamous cell carcinomas. In a study carried out in New Zealand, the proportion of p16+ OSCC increased from 24% during 1994-1999, to 76% during 2009-2014 (p<0.001)[10]. A study done in Sydney, Australia had 82.8% cases which showed p16 expression in OSCC with HPV association (p=0.007), and incidence of p16 expression significantly decreased with increasing tumor stage category (p=0.002)[11]. In a recent study in India, p16 positivity was noted in 86.66% of total OSCC samples (p=0.04)[12]. The rationale of the study was focused on evaluating the immuno-histochemical (IHC) frequency of human papillomavirus in oral squamous cell carcinoma specimens. This would help in assessment of the course and prognosis of the disease at time of initial diagnosis.

MATERIAL AND METHODS

This cross-sectional descriptive study was carried out at Armed Forces Institute of Pathology (AFIP), Rawalpindi from Jan 2017 to Jan 2018. A sample size of 30 was calculated using WHO sample size calculator keeping the confidence level % at 95%, anticipated population proportion at 10%, absolute precision required at 10%. After approval by ethical review committee, all specimens of oral squamous cell carcinoma detected by routine histopathology and immuno-histochemistry irrespective of age of patient, histological type and grade of the tumor, were included by non-probability, convenience sampling technique. Cases with inadequate biopsy were excluded from the study. Age and histo-pathological diagnosis was noted. All specimens were of patients of Pakistani origin. The data was analyzed by using computer software program SPSS version 24. The results of immuno-histo-chemistry were examined micro-scopically and verified by the single consultant to exclude observer bias. The reaction was considered positive when a chestnut-brown color would be seen in the nucleus and cytoplasm (figure). Two parameters were to be evaluated, percentage of p16-positive samples and their stratification based on age, gender and tumour size. Descriptive statistics like frequency were calculated for age.

RESULTS

A total of thirty (n=30) specimen of oral squamous cell carcinoma detected on histo-pathology were enrolled in the study. Immuno-histochemistry analysis was performed, and HPV was detected by applying p16, a surrogate marker of HPV, on formalin fixed and paraffin embedded sections. Immuno-histochemistry analysis revealed that there were 83.3% (n=25) samples which were positive for HPV-16. There were 63.3% (n=19) males with mean age of 61.8 years ± 13.9SD and 36.7% (n=11) females with mean age of 50.9 years ± 12.3SD (table-I). Mean

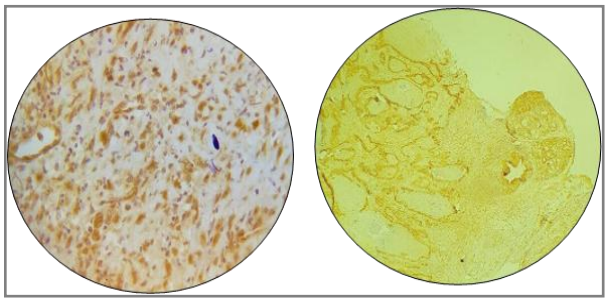

Figure: Immunohistochemistry slides showing HPV-16 nuclear positivity.

tumour size was 1.9 cm ± 1.4SD in the study sample (1.8 cm ± 1.1SD in males and 2.3 cm ± 1.8SD in females). Tumour size was <2cm in 73.3% (n=22) samples and >2cm in 26.7% (n=8) samples (table-II). Stratification with respect to gender (males and females), age (<60 years and >60 years) and tumor size (<2cm and >2cm) did not reveal any significant difference in HPV positivity. A p-value chi-square was 0.865, 0.317 and 0.460 respectively. Results are shown in table-III, IV & V.

DISCUSSION

Squamous cell carcinoma of the head and neck has long been regarded as a disease entity having a remarkable incidence worldwide and onerous prognosis[1]. Incidence of oral squamous cell carcinoma (OSCC) is approximately

14/100,000 accounting for 16% to 40% of all the malignancies[2]. In Pakistan, the prevalence of oral squamous cell carcinoma has reached 10% of all cancers[3]. Present study was designed to determine the frequency of HPV-16 in oral squamous cell carcinoma in our local population by immunohistochemical (IHC) analysis. Knowing the status of HPV-16 in OSCC at the time of initial diagnosis will add valuable information for the clinician predicting the course and prognosis of the disease. A total of thirty (n=30) specimen of oral squamous cell carcinoma detected on histopathology were enrolled in the study. Immunohistochemistry analysis was performed and HPV was detected by applying p16, a surrogate marker of HPV, on formalin fixed and paraffin embedded sections. Our results showed that there were 63.3% (n=19) males with mean age of 61.8 years ± 13.9SD and 36.7% (n=11) females with mean age of 50.9 years ± 12.3SD. Mean tumour size was 1.9 cm ± 1.4SD in the study sample (1.8 cm ± 1.1SD in males and 2.3 cm ± 1.8SD in females). Tumour size was <2cm in 73.3% (n=22) samples and >2cm in 26.7% (n=8) samples. Immuno-histochemistry analysis revealed that there were 83.3% (n=25) samples which were positive for HPV-16. No significant difference was observed for sub group analysis with respect to gender, age and tumor size (p<0.05). It has been reported that normal oral mucosa may act as a reservoir for new HPV infections and may acts as a source of recurring HPV-associated lesions. Reported prevalence of HPV in normal oral mucosa range from 0.6% to 81%. Other prevalence sites of HPVs include the epithelium of the vagina, vulva, penis, anal canal, cervix, perianal region[13,14]. We, however, did not study HPV peravlance in the present study. We suggest other studies to see other prevalence sites in our local population. Our results are similar with the studies on local population. In a study in local population[15], HPV was detected in 95 (68%) patients of oral squamous cell carcinoma, out of whom, 85 (90%) contained HPV16. We did not investigate for HPV18 in the present study. HPV-16 was found in 83.3% in our study, which is quite comparable with incidence reported by[15]. In a more recent study in local population[16],

Table-I: Gender/Age/Tumor size distribution in study sample.

Gender	Frequency	Percentage (%)	Mean age (years)	Tumor size (cm)
Males	19	63.3	61.8 ± 13.9	1.8 ± 1.1
Females	11	36.7	50.9 ± 12.3	2.3 ± 1.8
Total	30	100.0	57.8 ± 14.2	1.9 ± 1.4

Table-II: Different age/tumor size groups and HPV status in study sample.

Age group	Frequency	Percentage (%)
<60 Years	18	60.0
>60 Years	12	40.0
Total	30	100.0
Tumor size groups		
<2cm	22	73.3
>2cm	8	26.7
Total	30	100.0
HPV		
Positive	25	83.3
Negative	5	16.7
Total	30	100.0

DNA from oral rinse of 300 subjects (100 cases with OSCC and 200 controls [100 subjects with pre-malignant oral lesions and 100 normal persons]) were analyzed by both conventional and real time PCR using HPV consensus Gp5+/Gp6+ and HPV 16,18 specific primers. Out of 300 persons, 74/300 (25%) were found to be present at a higher stage and with large metastatic lymph nodes[17]. Our results showed males with OSCC are more significantly affected with HPV-16. We did not find significant association with age in our study. We also did not perform subgroup analysis based on clinical stage.

Table-III: HPV status in study sample (stratification w.r.t gender).

	HPV		Total	*p*-value chi-square
Gender	**Positive**	**Negative**		
Males	16	3	19	0.865
	84.2%	15.8%	100.0%	
Females	9	2	11	
	81.8%	18.2%	100.0%	
Total	25	5	30	
	83.3%	16.7%	100.0%	

Table-IV: HPV status in study sample (stratification w.r.t age).

	HPV			*p*-value chi-square
Age group	**Positive**	**Negative**	**Total**	
<60 years	14	4	18	0.317
	77.8%	22.2%	100.0%	
>60 years	11	1	12	
	91.7%	8.3%	100.0%	
Total	25	5	30	
	83.3%	16.7%	100.0%	

Table-V: HPV status in study sample (stratification w.r.t tumor size).

	HPV		Total	*p*-value chi-square
Tumor size	**Positive**	**Negative**		
<2cm	19	3	22	0.460
	86.4%	13.6%	100.0%	
>2cm	6	2	8	
	75.0%	25.0%	100.0%	
Total	25	5	30	
	83.3%	16.7%	100.0%	

infected with HPV. The distribution was: HPV16 was 4/100 (9%) from OSCC group while HPV 18 was 5/100(11%). We did not investigate for HPV 18 in the present study. HPV-16 was found in 83.3% in our study. The difference may be attributed to difference in sample size n=30 in present study versus n=300 in this study. It has been reported that patients with HPV-positive OSCC usually are younger and more often

It is also reported that low-risk HPV (HPV-6 and 11) appears to be closely associated with oral benign papillomatous lesions, while high-risk HPV (HPV-16,18) are in turn associated with malignant oral lesions. The reported rates of HPV DNA detection in OSCC range from 0% to 100%. This extreme variation is owing to difference in ethnicity, geographic locations to variations in methods used for detection of HPV (18,19).A

recent systematic review of total of 50 studies[20] analyzing the frequency of HPV in OSCC and other oral lesions reported that the frequency of HPV in OSCC varied from 0% to 80%. The HPV type most commonly detected in OSCC was HPV-16,18 with HPV-6,11 found in only a few studies. Whereas, HPV type found in oral benign lesions and papilloma was HPV-6 and 11. In another study conducted to determine the prevalence of HPV infection in OSCCs in 200 patients with OSCC and 68 normal controls. Authors reported that the prevalence of HPV of all types in the OSCC group was higher than in the control group (55/200 vs 2/68, OR=11.5, 95% CI=2.6-50.2). HPV16 and HPV18 were the main types detected, with HPV6 was the only low-risk type identified. High-risk HPV types HPV16 and HPV18 are prevalent in OSCC patients and may participate in the development of OSCC with traditional risk factors, tobacco and alcohol, possibly exerting synergistic effects[21]. We did not include benign oral lesions and we did not investigate other types other than HPV-16 in our study. In another metanalysis[22], authors observed that HPV may be a significant and independent risk factor for OSCC and by immunohistochemistry (IHC), most HPV-positive tumors show p16 overexpression. Another study revealed that genetic signatures of HPV-positive OSCC have been shown to be different from those of HPV-negative OSCC the expression of p53 and bcl-2 is not associated with HPV-positive OSCC and mutations in p53 are rarely seen in HPV-positive tumors compared with HPV-negative tumors[23]. Multiple pathways for HPV transmission to the oral cavity have been suggested. Oral HPV acquisition was found to be more positively associated with number of recent oral sex and open mouth kissing partners than with the number of vaginal sex partners[24]. We did not investigate these risk factors association in our study. Other reported risk factor in oropharyngeal cancers associated with HPV is Marijuana use and the risk increases with the intensity, duration, and cumulative years of marijuana smoking. Marijuana use may have a role in oropharyngeal cancers (OPCs) due to its immuno modulatory effect. Cannabinoids bind to the CB2 receptor of immune-modulatory cells in tonsillar tissue. This causes decreased immune response, reduced resistance to viral infections, and decreased antitumour actvity[25]. Toombak, a form of snuff, is associated with oral cancer in Sudan and it has been reported that 46% of oral squamous carcinomas were positive for HPV[26]. In contrast, a study[27] reported that HPV is detected less frequently in ex-smokers, current smokers, and tobacco chewers as compared to non-smokers and non-chewers. However, the association of HPV in subjects with greater than one lifetime sexual partner and in subjects with history of oral sex was present. Other studies showed that there is a wide array of assays used for detection of HPV in sample including PCR, ISH, IHC and Western blot analysis with PCR being the most widely used to estimate the HPV-DNA in samples[28]. HPV in saliva and oral exfoliated cells has been reported, but the sensitivity and specificity are too low, and the role of HPV detection in saliva is still uncertain. In summary, further research is needed in order to determine other prevalent sites for HPV in our local population. We also suggest risk factor analysis in a case control study with higher sampe size. Other low and high risk subtype also need to be analyzed in our local population. A comparative HPV profile in benign and malignant oral lesions is also needed. Standardization of a particular protocol for screening of patients with malignant and benign oral lesions for HPV is another area for future research in our settings.

CONCLUSION

A significant proportion of specimens of oral squamous cell carcinoma exhibited HPV-16 positivity in our population. No significant difference was noted across gender, age and tumor size.

LIMITATION OF STUDY

There were certain limitations in our study: i) We did not enroll the control group, ii) Our

sample size was relatively smaller for such kind of epidemiological studies, iii) We did not investigate other high and low risk subtypes of HPV in our study, and iv) We did not investigate other risk factors.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES

1. Khot KP, Deshmane S, Choudhari S. Human Papilloma Virus in Oral Squamous Cell Carcinoma. The Enigma Unravelled. Chin J Dent Res 2016; 19(1): 17-23.
2. Wang B, Zhang S, Yue K, Wang X-D. The recurrence and survival of oral squamous cell carcinoma: a report of 275 cases. CJCR 2013; 32(11): 614-8.
3. Markopoulos AK. Current Aspects on Oral Squamous Cell Carcinoma. Open Dent J 2012; 6: 126-30.
4. Hafed L, Farag H, Shaker O, El-Rouby D. Is human papilloma virus associated with salivary gland neoplasms? An in situ-hybridization study. Arch Oral Biol 2012; 57(9): 1194-9.
5. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, Brandsma J, et al. Molecular classification identifies a subset of human papillomavirus associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2013; 24(5): 736-47.
6. Zaravinos A. An updated overview of HPV-associated head and neck carcinomas. Oncotarget 2014; 5(12): 3956-69.
7. Machado J, Reis PP, Zhang T, Simpson C, Xu W, Perez-Ordonez B, et al. Low prevalence of human papillomavirus in oral cavity carcinomas. Head Neck Oncol 2010; 2(1): 6.
8. Izadi-Mood N, Asadi K, Shojaei H, Sarmadi S, Ahmadi SA, Sani S, et al. Potential diagnostic value of P16 expression in premalignant and malignant cervical lesions. J Res Med Sci 2012; 17(5): 428-33.
9. Brunner M, Koperek O, Wrba F, Erovic BM, Heiduschka G, Schoppper C, et al. HPV infection and p16 expression in carcinomas of the minor salivary glands. European archives of oto-rhino-laryngology : Head Neck Surg 2012; 269(10): 2265-9.
10. Kwon HJ, Brasch HD, Benison S, Marsh RW, Itinteang T, Titchener GW, et al. Changing prevalence and treatment outcomes of patients with p16 human papillomavirus related oropharyngeal squamous cell carcinoma in New Zealand. Br J Oral Maxillofac Surg 2016; 54(8): 898-903.
11. Satgunaseelan L, Virk SA, Lum T, Gao K, Clark JR, Gupta R. p16 expression independent of human papillomavirus is associated with lower stage and longer disease-free survival in oral cavity squamous cell carcinoma. Pathology 2016; 48(5): 441-8.
12. Patil S, Rao RS, Amrutha N, Sanketh DS. Analysis of human papilloma virus in oral squamous cell carcinoma using p16: An immunohistochemical study. J Int Soc Prevent Communit Dent 2014; 4(1): 61-6.
13. D'Souza G, Agrawal Y, Halpern J, Bodison S, Gillison ML. Oral Sexual Behaviors Associated with Prevalent Oral Human Papillomavirus Infection. J Infect Dis 2009; 199(9): 1263-9.
14. Ragin C, Edwards R, Larkins-Pettigrew M, Taioli E, Eckstein S, Thurman N, et al. Oral HPV Infection and Sexuality: A Cross-Sectional Study in Women. Int J Mol Sci 2011; 12(6): 3928-40.
15. Ali SMA, Awan MS, Ghaffar S, Salahuddin I, Khan S, Mehraj V, et al. Human papillomavirus infection in oral squamous cell carcinomas: correlation with histologic variables and survival outcome in a high risk population. Oral Surgery 2008; 1(2): 96-105.
16. Zil e R, Baig S, Zaman U, Lucky MH. Human Papilloma Virus 16/18: Fabricator of Trouble in Oral Squamous Cell Carcinoma. Int J Infect Dis 2018; 69: 115-19.
17. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007; 356(19): 1944-56.
18. Prabhu SR, Wilson DF. Human papillomavirus and oral disease - emerging evidence: A review. Aust Dent J 2013; 58(1): 2-10.
19. Campisi G, Panzarella V, Giuliani M, Lajolo C, Di Fede O, Falaschini S, et al. Human papillomavirus: its identity and controversial role in oral oncogenesis, premalignant and malignant lesions (review). Int J Oncol 2007; 30(4): 813-23.
20. Gupta S, Gupta S. Role of human papillomavirus in oral squamous cell carcinoma and oral potentially malignant disorders: A review of the literature. Indian J Dent 2015; 6(2): 91-8.
21. Gan LL, Zhang H, Guo JH, Fan MW. Prevalence of human papillomavirus infection in oral squamous cell carcinoma: a case-control study in Wuhan, China. Asian Pac J Cancer Prev 2014; 15(14): 5861-5.
22. Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics 2001; 91(6): 622-35.
23. Oliveira MC, Soares RC, Pinto LP, Souza LB, Medeiros SR, Costa Ade L. High-risk human papillomavirus (HPV) is not associated with p53 and bcl-2 expression in oral squamous cell carcinomas. Auris Nasus Larynx 2009; 36(4): 450-6.
24. Kreimer AR, Alberg AJ, Daniel R, Gravitt PE, Viscidi R, Garrett ES, et al. Oral human papillomavirus infection in adults is associated with sexual behavior and HIV serostatus. J Infect Dis 2004; 189(4): 686-98.
25. Westra WH. The Changing Face of Head and Neck Cancer in the 21st Century: The Impact of HPV on the Epidemiology and Pathology of Oral Cancer. Head Neck Pathol 2009; 3(1): 78-81.
26. Babiker AY, Eltom FM, Abdalaziz MS, Rahmani A, Abusail S, Ahmed HG. Screening for high risk human papilloma virus (HR-HPV) subtypes, among Sudanese patients with oral lesions. IJCEM 2013; 6(4): 275-81.
27. Herrero R, Castellsague X, Pawlita M, Lissowska J, Kee F, Balaram P, et al. Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. J Natl Cancer Inst 2003; 95(23): 1772-83.
28. Braakhuis BJ, Brakenhoff RH, Meijer CJ, Snijders PJ, Leemans CR. Human papilloma virus in head and neck cancer: the need for a standardised assay to assess the full clinical importance. Eur J Cancer 2009; 45(17): 2935-9.