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A STUDY OF ROLE OF HPV 16 IN ORAL MALIGNANCY USING HPV 16 and p16 IMMUNOHISTOCHEMISTRY MARKERS

Oral Pathology

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ABSTRACT

Background: Head and neck cancers account for 4% of all cancers globally. HPV is recognized as carcinogenic infectious agents not only in cervical cancer but also in head and neck squamous cell cancer (HNSCC), especially oropharyngeal cancer. This study aims at establishing a correlation of HPV with oral malignancies and to establish the role of immunohistochemistry (IHC) to detect HPV positive cases. **Methods:** 127 Cases of oral malignancy were taken from the Department of histopathology at a tertiary care centre. We studied the cases in terms of sub site, risk factors, gross presentation, histology grade, and IHC markers for p16 and HPV 16. **Result:** In our study we found that 33 males (82.5%) and 07 females (17.5%) were HPV positive, 30 males (88.2%) and 04 females (11.7%) were P16 positive. Of these 40 patients who tested positive for HPV, 26 patients tested positive for p16. Most of the cases of HPV positive SCC were well differentiated (17.3%) and moderately differentiated (13.3%) SCC. **Conclusion:** The results of our study have demonstrated that HPV16 plays a significant role in carcinogenesis in head neck cancers. HPV positive tumours have distinct biological profile in comparison to the HPV negative counterparts in Indian population also. Since the clinical outcomes also vary with HPV 16 status of the tumour, HPV testing should be routinely performed with HPV16 and p16 immunohistochemistry in cases of head and neck squamous cell cancers.

KEYWORDS

Human Papilloma virus (HPV), Squamous cell carcinoma (SCC), Immunohistochemistry (IHC), Head & neck cancer & P16.

INTRODUCTION

Head and neck cancers account for 4% of all cancers globally.[1] It is second most common cancer in India after carcinoma breast while it is most common cancer in India in males. Traditionally head and neck cancers have been associated with smoking and alcohol. [2] The effect of alcohol and smoking is synergistic. [2]The association between human papilloma virus (HPV) and cervical cancer was made already in the 1970's; although; it was only gradually accepted by the scientific community.[3] Today, these viruses are recognized as carcinogenic infectious agents not only in cervical cancer but also in head and neck squamous cell cancer (HNSCC), especially oropharyngeal cancer.[4] It has been postulated that patients with HPV-associated oral malignancies have a better response to treatment than their stage-matched non HPV-related oral malignancies. [5] Thus, HPV–related oral malignancies define a unique population of patients with distinct biology that should be treated separately from non-HPV-related oral malignancies. P16 status is also reported as an independent prognostic factor for disease free survival.[6] In this study we aim to establish a correlation of HPV with oral malignancies and to establish the role of immunohistochemistry (IHC) to detect HPV positive cases.

MATERIAL AND METHOD

This study was a prospective, single centre, cross sectional study. 127 Cases of oral malignancy were taken from the department of histopathology at a tertiary care centre. The tissues were stained separately for H&E and IHC. Standard technique was used for H&E staining. For IHC biogenex kits were used for HPV16 and P16. Tissue mounted on poly lysine coated slides was stained for HPV16 and p16. Slides for HPV 16 were evaluated based on nuclear stain as positive or negative. For P16 an index of positivity was calculated by counting labelled cells in 1000 counted cells in 40x magnification. Score of 0 (<10% stained cells); 1 (10-15% stained cells); and 2 (>50% stained cells) was given. The statistical data was recorded in SPSS software. Comparison of the qualitative variables was done using chi-square test. The data was expressed in terms of percentage and cross tabulation.

OBSERVATION AND RESULTS

Out of 127 patients 106 patients were males and 21 patients were females. We studied the cases in terms of sub site, risk factors, gross presentation, histology grade, and IHC markers for p16 and HPV 16. We recorded detailed history of all the patients in accordance with case pro-forma after obtaining written informed consent. The mean age of presentation was 50.08 years +/-13.82 SD. Among males mean age was 50.46 years and 57.82 years in females. We found that 33 males and 07 females were HPV positive, 30 males and 04 females were P16 positive. The mean age for HPV positive patients in males was 53.54 years (p-0.946) and 51.14 years (p-0.988) in females. 96 patients had oral cavity malignancy, 14 had cancer larynx, 13 had cancer oropharynx and 04 had cancer hypopharynx. The correlation of oropharynx and HPV positivity was significant with p value=0.0065 (<0.5CI). Most common histology among our patients (63) was well differentiated squamous cell carcinoma. 48 patients had moderately differentiated squamous cell carcinoma. Poorly differentiated (09 cases), verrucous carcinoma (05) and oral intraepithelial neoplasm (02) were other histological type found in our study. Most of the cases of HPV positive SCC were well differentiated (17.3%) and moderately differentiated (13.3%) SCC. One case of HPV positive SCC was poorly differentiated while OIN or Verrucous carcinoma was not found on histopathology in HPV positive cases. On HPV immune-staining we found that 33 males and 07 females were HPV positive. Of these 40 patients who tested positive for HPV, 26 patients tested positive for p16. The IHC for p16 was assessed on the basis of index of positivity (IP) and intensity (IS). IP was 1+ for 26 patients and 2+ for 08 patients whereas, IS was 1+ for 28 patients and 2+ for 05 patients. In our study we found that of the 34 patients were p16 positive, 20 cases were of WDSCC with 14 patients having IP of 1+ and 06 patient had IP of 2+. 14 patients had MDSCC of which 12 had IP score of 1+ and 02 patient had IP score of 2+. The IS score for WDSCC was 1+ for 17 cases and 2+ for 02 case whereas, for MDSCC cases 11 patients had IS score of 1+ and 03 patients had IS score of 2+(Figure 1-4). The detailed analysis of HPV 16 and p16 in comparison to HPV negative cases have been represented in table 1 & 2.

Table 1 : Detailed Analysis Of P16 Positive And Negative Cases

Parameter		P16positive cases (n=34)	P16 negative cases (n=93)
Gender	Males	M- 30 (88.2%)	M- 76 (81.7%)
	Females	F- 04 (11.7%)	F- 17 (18.2%)
Mean age		55.17 years	46.63 years
Risk factors	Tobacco chewing	09 (26.4%)	26 (27.9%)
	Tobacco chewing and smoking	18 (52.9%)	49 (52.6%)

	Tobacco and alcohol	05 (14.7%)	08 (8.6%)
	None	02 (5.8%)	10 (11.5%)
Sites	Oral cavity	22 (55%)	74 (85.05%)
	Hypopharynx	02 (5%)	02 (2.3%)
	Larynx	07 (17.5%)	07 (8.05%)
	Oropharynx	09 (22.5%)	04 (4.6%)
Histopathology	OIN I/II/III	00	02 (2.3%)
	WDSCC	20(58.8%)	43(46.2%)
	MDSCC	14 (41.1%)	34 (36.5%)
	PDSCC	00	09 (9.6%)
	VC	00	05 (5.7%)
Gross presentation	Proliferative growth	17 (50.0%)	29 (31.1%)
	Ulcer	17 (50.0%)	57 (61.2%)
	White patch	00	07 (6.3%)

Table 2 : Detailed Analysis Of HPV16 & P16 Positive And Negative Cases

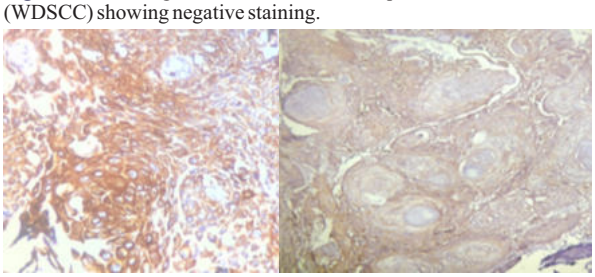
Parameter		**HPV & P16positive cases (n=26)**	**HPV & P16 negative cases (n=101)**
Gender	Males	M-22 (84.61%) (p value-0.9656)	M- 84 (83.16%)
	Females	F- 04 (15.38%) (p value-0.9020)	F- 17 (16.83%)
Mean age		56.46 years	45.34 years
Risk factors	Tobacco chewing	04 (15.38%) (p value-0.3009)	31 (30.69%)
	Tobacco chewing and smoking	15 (57.69%) (p value-0.8024)	52 (51.48%)
	Tobacco and alcohol	05 (14.7%) (p value-0.2612)	08 (8.6%)
	None	02 (5.8%) (p value-0.7951)	10 (11.5%)
Sites	Oral cavity	12 (46.15%) (p value-0.1844)	84 (83.16%)
	Hypopharynx	01 (3.84%) (p value-0.8604)	03 (2.97%)
	Larynx	06 (23.07%) (p value-0.1588)	08 (7.92%)
	Oropharynx	07 (26.92%) (p value-0.05)	06 (5.94%)
Histopathology	OIN I/II/III	00	02 (2.3%)
	WDSCC	16 (61.53%) (p value-0.5411)	47 (46.53%)
	MDSCC	10 (38.46%) (p value-0.9659)	38 (37.62%)
	PDSCC	00	09 (9.6%)
	VC	00	05 (5.7%)
Gross presentation	Proliferative growth	15 (57.69%) (p value-0.6101)	31 (30.69%)
	Ulcer	11 (42.30%) (p value-0.4084)	63 (62.37%)
	White patch	00	07 (6.3%)

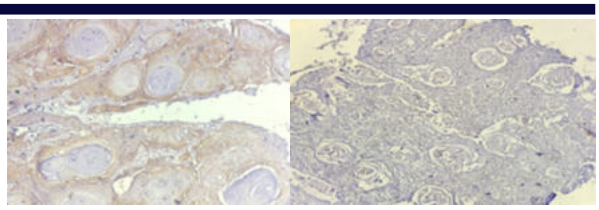
Fig1: HPV 16 staining in Moderately differentiated squamous cell carcinoma (MDSCC) showing nuclear positive staining

Fig 2: HPV 16 staining in Well differentiated squamous cell carcinoma (WDSCC) showing nuclear positive staining

Fig 3: P16 in Well differentiated SCC (WDSCC) showing high index of positivity (IP) score 2 and strong staining intensity (IS) score2

Fig 4: P16 staining in Well differentiated squamous cell carcinoma (WDSCC) showing negative staining.





DISCUSSION

This study was aimed to study the role of HPV 16 in oral malignancies using HPV 16 and p16 IHC markers. It has been observed by Mendenhall WM et al.(2009)[7]that patients with HPSCC present at younger age than HNSCC. It has been observed that the mean age of our study is approximation to the study conducted by Ang KK et al. (2010) [8] and it was higher in Christopher A. Maroun et al. (2020)[9] In our study mean age is 50.08year +/- 13.82 years SD. Among males mean age was 50.46 years and 57.82 years in females. The mean age for HPSCC was 53.12 years in our study in comparison to 48.68 years in HNSCC. Head and neck malignancy are more common in males. Age standardised incidence rate is 13.9 per 100 000 in males and 4.3 per 100 000 in females in India.[10] According to Globocon 2018 data oral SCC is less common in females worldwide however in India incidence is more among females due to tobacco abuse among in low socio-economic strata.[11] In present study there were 82.5% males and 17.5% females HPV Positive which is comparable with the studies of Nasrollah Saghravanian et al. (2015)[12] where it was 53.3% & 46.7% respectively. According to Patel SC et al,(2011) there is increasing incidence of oral SCC in non-smoking females in western countries that doesn't appear to be driven by HPV exposure.[13]

There are multiple studies done to study immunoexpression of P16 in oral SCC over the last decade. The immune expression of P16 observed in our study was almost similar to the Mark E Zafereo et al (2016). The results varied from the lowest i.e. 17.2% in a study conducted by N Sgaramellaet. al.(2015) to 62% in a study conducted by AdrijaPathak et.al.[14] (2019) respectively. In our study we found that 26.77% patient were P16 positive. The correlation of HPV with oropharynx cancer and nasopharynx cancer has been studied thoroughly. In the study by Chaturvedi AK et al (2011) stated that from 1998 to 2004 there has been 225% increase in HPV related head and neck cancers, however in a study by Naeem B et al the prevalence of HPV related Head and neck SCC in Asia was reported to be 10.42% [15][16] . In our study we found that 96 patients had oral cavity malignancy, 14 had cancer larynx, 13 had cancer oropharynx and 04 had cancer hypopharynx, of these we found that of the 34 patients (26.77%) tested positive for both HPV and p16, 30 patients (88.23%) were male and 04 patient was female, suggesting that HPV was implicated in pathogenesis of SCC in such cases.

More than 90% of cancers of head and neck are squamous cell carcinoma.[17] Squamous cell carcinoma arises from the regions lined by stratified squamous epithelium. The classic malignancies are typically preceded by presence of premalignant lesions. The gross appearance of early lesions can either be raised, firm, pearly plaques or irregular, roughened or verrucous areas of mucosal thickening appearing as white plaques. In our study we divided the gross features among three types namely; proliferative growth, ulcer and white patch. The most common presentation in our study was ulcer seen in 58.27% patients. 46 patients presented with proliferative growth and 07 patients presented with a white patch. Among HPV positive cases 18 cases presented with ulcer and 22 patients presented as proliferative growth. In our study we classified all patients histologically according to border's grading. We classified patients into five major subtypes i.e. Oral Intraepithelial Neoplasm (OIN I/II/III), well differentiated SCC, moderately differentiated SCC, poorly differentiated SCC and verrucous carcinoma. In a study by Chernock RD et al (2011) and Lewis JS et al (2016), they described the difference in histological difference between HNSCC and HPSCC.[16][17] They stated that classically, most oropharynx cancers are well differentiated and often show keratinization, which is most often seen in elderly males and associated with smoking and alcohol, and were HPV negative. On the contrary to this HPV positive cancers are seen among 40- 55 years in males, are non-keratinizing with less tobacco and alcohol abuse.

In a study conducted by Gaurav Prahalhad Agrawal et al (2013)[18] the prevalence rate of HPV 16 & P16 positive cases on the basis of histopathological subtypes was reported as OIN I/II/III (10%), WDSCC (44%), MDSCC (14.28%), PDSCC (14.28%) and VC (0%).

These findings were consistently seen in our study. Most common histology among HPV &P16 patients was WDSCC (61.54%). 38.46% patients in our study were MDSCC. No positive cases were observed in OIN, PDSCC and VC. HPV positive patients 55% patients were having oral cavity lesion and 22.5% patients had oropharynx lesion. Most of the HPSCC lesions were moderately differentiated. According to study conducted by Mandenhall WM et al (2009), Licitra L et al (2006)& ANG KK et al (2010) it has been proved that overall survival in HPV positive SCC is better than HPV negative counterparts.[19][20] HPV positive cases have better response to 5-florouracil and cisplatin based chemotherapy.[21][22] Our study was underpowered to study the clinical and biological behaviour of HPV positive head and neck cancers. Treatment outcomes were not included in our study. During the last decade it has been well established that HPV associated head and neck cancers have a distinct biological behaviour. The correlation between HPV and, head and neck cancer has been studied thoroughly in the western population. Above findings from our study demonstrates that in Indian patients with head and neck cancers HPV 16 has a significant role to play in carcinogenesis. HPV positive cases have a varied epidemiological profile, risk factors and histology.

CONCLUSION

In a prospective, single centre, cross sectional study conducted on histologically proven 127 patients of head and neck squamous cell cancers we applied HPV16 and p16 immuno-staining. The results of our study have demonstrated that HPV16 plays a significant role in carcinogenesis in head neck cancers. HPV positive tumours have distinct biological profile in comparison to the HPV negative counterparts in Indian population also. Since the clinical outcomes also vary with HPV 16 status of the tumour, HPV testing should be routinely performed with HPV16 and p16 immunohistochemistry in cases of head and neck squamous cell cancers. Our study was limited to study the clinical and biological behaviour of HPV positive head and neck cancers. Treatment outcomes were not included in our study. To establish the prognostic significance of HPV in oral malignancies more detailed large cohort multi-centric clinical trials should be advised.

REFERENCES:

[1]. American Cancer Society. *Cancer Facts and Figures 2017*. Atlanta, GA: American Cancer Society;2017

[2]. Blot WJ, McLaughlin JK, Winn DM, Austin DF, Greenberg RS, Preston-Martin S, Bernstein L, Schoenberg JB, Stemhagen A, Fraumeni JF Jr. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res. 1988 Jun 1;48(11):3282-7. PMID: 3365707.

[3]. zurHausen H. Papillomaviruses in the causation of human cancers: a brief historical account. Virology. 2009;384:260–265.

[4]. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, Zahurak ML, Daniel RW, Viglione M, Symer DE, Shah KV, Sidransky D. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. J Natl Cancer Inst. 2000 May 3;92(9):709-20. doi: 10.1093/jnci/92.9.709. PMID: 10793107.

[5]. Mannarini L, Kratochvil V, Calabrese L, et al. Human Papilloma Virus (HPV) in head and neck region: review of literature. *ActaOtorhinolaryngol Ital*. 2009;29(3):119-126

[6]. Meng HX, Miao SS, Chen K, et al. Association of p16 as Prognostic Factors for Oropharyngeal Cancer: Evaluation of p16 in 1470 Patients for a 16 Year Study in Northeast China. *Biomed Res Int*. 2018;2018:9594568. Published 2018 Sep 17

[7]. Siddiqui MS, Chandra R, Aziz A, Suman S. Epidemiology and histopathological spectrum of head and neck cancers in Bihar, a state of Eastern India. Asian Pac J Cancer Prev. 2012;13(8):3949-53.

[8]. Mendenhall WM, Logan HL. Human papillomavirus and head and neck cancer.Am J ClinOncol. 2009 Oct;32(5):535-9.

[9]. Sinha P, Logan HL, Mendenhall WM. Human papillomavirus, smoking, and head and neck cancer. *Am J Otolaryngol*. 2012;33(1):130-136.

[10]. Patel SC, Carpenter WR, Tyree S, et al: Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. J ClinOncol 29:1488, 2011

[11]. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J ClinOncol*. 2011;29(32):4294-4301.

[12]. Bukhari, N., Joseph, J., Hussain, S., Khan, M., Wakim, M., Yahya, E., Arif, A., Saleem, A., Sharif, N. Prevalence of Human Papilloma Virus Sub Genotypes following Head and Neck Squamous Cell Carcinomas in Asian Continent, A Systematic Review Article. Asian Pacific Journal of Cancer Prevention, 2019; 20(11): 3269-3277.

[13]. Mishra GA, Pimple SA, Shastri SS. An overview of the tobacco problem in India. *Indian J Med PaediatrOncol*. 2012;33(3):139-145.

[14]. De Felice F, Tombolini V, Valentini V, et al. Advances in the Management of HPV-Related Oropharyngeal Cancer. *J Oncol*. 2019;2019:9173729. Published 2019 Apr 14.

[15]. Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. J ClinOncol. 2011;29(32):4294-4301.

[16]. Bukhari, N., Joseph, J., Hussain, S., Khan, M., Wakim, M., Yahya, E., Arif, A., Saleem, A., Sharif, N. Prevalence of Human Papilloma Virus Sub Genotypes following Head and Neck Squamous Cell 106 Carcinomas in Asian Continent, A Systematic Review Article. Asian Pacific Journal of Cancer Prevention, 2019; 20(11): 3269-3277.

[17]. Lewis, J. Morphologic diversity in human papillomavirus-related oropharyngeal squamous cell carcinoma: Catch Me If You Can!. Mod Pathol 30, S44–S53 (2017)

[18]. GauravPralhadAgrawal, PriyaShirish Joshi, AnshitaAgrawal, "Role of HPV-16 in Pathogenesis of Oral Epithelial Dysplasia and Oral 107 Squamous Cell Carcinoma and Correlation of p16INK4A Expression in HPV-16 Positive Cases: An Immunohistochemical Study", International Scholarly Research Notices, vol. 2013, Article ID 807095, 7 pages, 2013.

[19]. Licitra L, Perrone F, Bossi P, Suardi S, Mariani L, Artusi R, Oggionni M, Rossini C, Cantù G, Squadrelli M, Quattrone P, Locati LD, Bergamini C, Olmi P, Pierotti MA, Pilotti S. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J ClinOncol. 2006 Dec 20;24(36):5630-6.

[20]. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, NguyenTân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R, Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med. 2010 Jul 1;363(1):24-35.

[21]. De Felice F, Tombolini V, Valentini V, et al. Advances in the Management of HPV-Related Oropharyngeal Cancer. J Oncol. 2019;2019:9173729. Published 2019 Apr 14.

[22]. Wierzbicka M, Szyfter K, Milecki P, Składowski K, Ramlau R. The rationale for HPV-related oropharyngeal cancer de-escalation treatment strategies. ContempOncol (Pozn). 2015;19(4):313-322.