ORIGINAL ARTICLE

ASSOCIATION OF HIGH RISK HUMAN PAPILLOMA VIRUS (HPV-16/18) WITH P16 PROTEIN IN ORAL PREMALIGNANT LESIONS AND ORAL SQUAMOUS CELL CARCINOMA

Saadia Akram[1], Asma Shabbir[1], Talat Mirza[2]
[1]Department of Pathology, Sindh Medical College, Jinnah Sindh Medical University, Karachi,
[2]Dean of Research Department, Dr. Ziauddin Hospital and University, Karachi, Pakistan.

ABSTRACT

Background: Oral cancer is a major problem globally. The strong causal association with tobacco, prevalent in Pakistan makes it imperative to know the role of molecular events in oral oncogenesis. We aim to evaluate high risk HPV 16/18 and p16 in oral premalignant lesions (OPL) and oral squamous cell carcinoma (OSCC). We further analyze the association between high risk human papilloma virus (HR-HPV16/18) and p16 in OPL and OSCC.

Methods: A total of 100 OSCC and 50 OPL cases were included. Demographic data along with habitual exposure to smoked and chewable tobacco, betel and gutka etc., was noted. We evaluated p16 in OPL and OSCC by immunohistochemistry, HPV was detected by polymerase chain reaction. Data was entered and analyzed using SPSS 21. Chi square and Fisher exact were applied to determine the association of HPV and p16 with different variables.

Results: Out of 50 OPL, 14% were positive and 86% were negative for p16 whereas out of 100 OSCC, 18% were positive and 82% were negative. Out of 50 OPL, HPV was detected in 6% whereas out of 100 OSCC, 15% were positive. Highly significant co expression of HPV with p16 was observed in all 15 (100%) HPV positive OSCC cases (p = 0.001). However, 3 out of 18 cases, which showed p16 expression, did not show HPV infection.

Conclusion: Role of p16 as a surrogate marker for HPV in OSCC can be supported in the present study. Moreover a Chemical carcinogen like tobacco is considered as major associative risk factor with p16 and HPV in concert.

Keywords: Human Papilloma Virus (16/18); P16; Oral Premalignant Lesions; Oral Squamous Cell carcinoma.

Corresponding Author:
Dr. Asma Shabbir
Department of Pathology,
Sindh Medical College,
Jinnah Sindh Medical University.
Karachi, Pakistan.
Email: drasma52@gmail.com
doi.org/10.36283/PJMD8-4/002

INTRODUCTION

Oral cancer ranks as the sixteenth most common malignancy in the world and 12th most common cancer in Asia[1,2]. It is highly prevalent in South Asian countries like Bangladesh, India, Pakistan and Sri Lanka, where one-third of all the cancers reported are oral cancers[2]. Shaukhat Khanum Memorial Cancer Hospital and Research Centre (SKMCHRC) reported oral cancer as 7th most common cancer in Pakistan for the year 2017[3].

Oral cancers are multifactorial in origin with contribution of individual's genetic makeup and environmental influences. They are usually preceded by oral premalignant lesions (OPL) which are histologically classified as dysplasia (mild, moderate, severe), leading to carcinoma in situ[4]. A strong relationship of OPL and oral cancer has been established with conventional risk factors including chewable tobacco mixtures, betel quid, areca nut, smoking and their combinations[5-7].

Human Papilloma Virus (HPV) is now recognized as an established risk factor for oral squamous cell carcinoma (OSCC), particularly in the oropharynx. HPV association with OSCC and OPL shows a wide variation, ranging from 0-100% in different studies around the world[8]. The p16 protein is a tumor suppressor gene located on chromosome 9p21. It is expressed at a low level in normal cells. Loss of p16 expression is noted in early stages of carcinogenesis[9-11]. Moreover, loss of p16 is also observed in patients of OSCC with exposure to conventional risk factors, through mechanisms of hypermethylation of promoter region, homozygous deletion or loss of heterozygosity[11,12]. Interestingly, literature revealed p16 overexpression in tumors with biologically active HPV infection. Normally, pRB represses p16 transcription, but, in HPV infection, the E7 protein of HPV causes inactivation of pRB, which in turn leads to overexpression of p16[13].

The relationship between HPV positivity and p16 immunoreactivity has given a prospect for p16 over-expression to find a place as a surrogate marker for HPV detection. However, their correlation appears to be more significant in head and neck squamous cell carcinoma (HNSCC) as compared to primary OSCC[11,14]. Studies have also reported that p16 over-expression can also be exhibited by pathways other than HPV E7 protein[13]. Hence, the impact of p16 overexpression in HPV infected OSCC as a surrogate marker needs to be explored. In this study, we aimed to determine the expression of p16 in OPL and OSCC, detect HR-HPV 16/18 in OPL and OSCC and analyze the correlation between p16 and HPV 16/18.

METHODS

The study was conducted at Dow Diagnostic Research and Reference Laboratory (DDRRL), Dow University of Health Sciences (DUHS), Karachi after ethical approval of Institutional Review Board (Reference number: Ph-D-13/ERB-64/DUHS-08). A total of 150 biopsies from oral cavity (100 cases of OSCC and 50 cases of OPL) were collected from Otolaryngology ward of Civil Hospital, Karachi and Dr. Ishrat ul Ebad Khan Institute of Oral Health Sciences (DIKIOHS) at DUHS after informed consent by the patients. Clinico-pathological parameters of the patients were then documented. Personal habits and addictions especially betel quid chewing, tobacco chewing and smoking were evaluated in detail as risk factors for OPL and OSCC. Furthermore, 25 cases of normal mucosal samples obtained from molar extraction tissue and 25 cases of hyperplasia were included for histological and molecular comparison.

Subsequent to biopsy, routine grossing and hematoxylin and eosin (H and E) staining was performed. Histopathological grading of OPL was done as mild, moderate and severe dysplasia by Barnes classification[4].

According to the World Health Organization (WHO) criteria, histopathological grading of OSCC was done as grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated). As per the American Joint Committee on Cancer Staging (stage I - stage IV), clinical staging of all the cases of OSCC were recorded[15]. In order to investigate the expression of p16 in OPL and OSCC, all cases were subjected to conventional immunohistochemistry protocol. Sections were deparaffinized, dehydrated and antigen retrieval of antigen was carried out. Mouse primary monoclonal human antibody against p16 (5A8A, Abbiotec) were incubates with treated sections for1 hour in the moisturizer chamber. Secondary antibody (HRP conjugated anti-rabbit antibody) (Abbiotec) containing A as amplifier and solution B as polymer were used. For all read outs, staining was controlled by using known p16 expressing normal mucosal samples and cases of hyperplasia. Two experienced pathologists evaluated the immune-stained slides. All the cases were assessed for diffuse nuclear and cytoplasmic stain by p16.In order to quantify p16 stain; a semi-quantitative method was used by multiplying intensity and percentage of cells affected[16].

The intensity was depicted as follows: i. Weak =1, ii. Moderate = 2 and iii. Strong = 3. The percentage of cells stained was graded as follows: i. Absent staining = 0, ii. 0-10% cells stained = 1, iii. 11-50% cells stained = 2, iv. 51 - 80% cells stained = 3 and v. 81 - 100% cells stained = 4. Final score of 0-12 was obtained by multiplying the two variables. A score of 4 or above was taken as positive.

All the study samples were collected and cut into five microns' thickness. The QIA amp DNA tissue kit (Invitrogen Life Technologies, Carlsbad, USA) (Qiagen, Valencia, CA) was used to extract DNA from the tissue sections according to the manufacturers instruction. HPV genotype was analyzed through Real time PCR by using 12.5 µl of extracted DNA. The quantitative genotyping of HPV-16 and HPV-18 in isolated DNA was done by using real-time PCR kit (HPV 16/18 Real TM Quant, Sacace Biotechnologies, Italy) by real time PCR machine (Smart Cycler II, Cephied, USA). The principal of assay was based on two major processes viz isolation of DNA from specimens and multiplex Real Time amplification. Results of HPV16 DNA amplification were detected on the FAM/green channel, amplification results of HPV-18 DNA were detected on the Rox/orange channel and β-globin gene used as internal control detected on the Joe/HEX/yellow channel. For the interpretation of the results, software of Smart Cycler Real-time PCR was used to obtain the fluorescence curve with the threshold line generated by the system in real-time.

Using SPS version 21 for data were recording of different variables including age, gender, grade, type, stage, HPV expression and p16. Descriptive statistics were applied to know the frequency of the given variables. In order to investigate the association of p16 and HPV with different variables and to find the association between p16 expression and HPV, later, Chi square or Fisher's exact were applied and a *p*-value < 0.05 was considered as significant.

RESULTS

The study included a total of 50 cases of oral premalignant lesions (OPL) with the mean age of the patients being 40.22 ± 9.66 years. Thirty-five (70%) out of these were males while 15 (30%) were females. Majority of OPL cases, 46 (92%) were habituated to risk factors. Check, 21 (42%), was the commonest site of OPL in our series. Clinically, leukoplakia, 40 (80%) was the most commonly diagnosed OPL. Histologically, a total of 25 (50%) patients had mild dysplasia, 16 (32%) had moderate dysplasia and 9 (18%) had severe dysplasia.

The series comprised a total of 100 cases of oral squamous cell carcinoma (OSCC). Mean age of the patients was 47.84 ± 12.18 years. It included a total of 74 (74%) males and 26 (26%) females. 91(91%) patients were habituated with risk factors. Cheek 50 (50%) was the commonest site of OSCC lesion. Histologically, 48 (48%) patients with grade I, 37 (37%) with grade II and 15 (15%) with grade III tumors were reported. Six (6%) cases were of stage I, 20 (20%) cases of stage II, 35 (35%) cases of stage III and 39 (39%) cases of stage IV cancer.

Out of total 50 cases of OPL, 43 (86%) cases revealed loss of p16 expression while 7 (14%) cases showed over expression of p16. No significant association was seen between both, loss of p16 and over expression of p16 with different variables including age, gender, site, risk factors, clinical and histological presentation.

Out of total 100 cases of OSCC, lack of p16 expression was seen in 82 (82%) cases whereas over expression of p16 was noted in 18 (18%) cases. A highly significant relationship between p16 negative cases was evident with tumor differentiation, most cases were seen in grade I and grade II malignancy ($p = 0.000$). Significant association was also seen between p16 negative cases and habituated risk factors ($p = 0.002$). No significant association was detectable between loss of p16 expression and age, gender, site and stage of tumor. Since, p16 overexpression showed no significant association with age, gender, site, risk factors, grade and stage of the tumors (Table 1) (Figure 1).

Table 1: Correlation of p16 with clinic-pathological parameters in OSCC.

Parameters	P16		Total	Chi sq *p*-value
	Negative (82%)	Positive (18%)		
Age Groups				
21-30years	6(66.7%)	3(33.3%)	9	0.3684
31-40years	20(83.3%)	4(16.7%)	24	
41-50years	30(88.2%)	4(11.8%)	34	
51-60years	18(81.8%)	4(18.2%)	22	
61-70years	6(75%)	2(25%)	8	
71-80years	2(66.7%)	1(33.3%)	3	
Gender				
Male	58(78.4%)	16(21.6%)	74	0.112
Female	24(92.3%)	2(07.7%)	26	
Risk Factors				

No Habits	8(88.9%)	1(11.1%)	9	0.002*
Tobacco Smoking	9(56.3%)	7(43.8%)	16	
BQ/Tobacco chewing	44(95.7%)	2(04.3%)	46	
Smoking with BQ/ Tobacco chew	21(72.4%)	8(27.6%)	29	
Site				
Cheek	40(80%)	10(20%)	50	0.657
Tongue	21(87.5%)	3(12.5%)	24	
Lip	5(71.4%)	2(28.6%)	7	
Alveolus	8(88.9%)	1(11.1%)	9	
Palate	4(66.7%)	2(33.3%)	6	
Floor	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	

Significant at 0.05, **Significant at 0.01, ~Chi-sq Test P-value with More than 20% Cells Proportion * Test: Chi-square for Association, Fisher Exact Test.

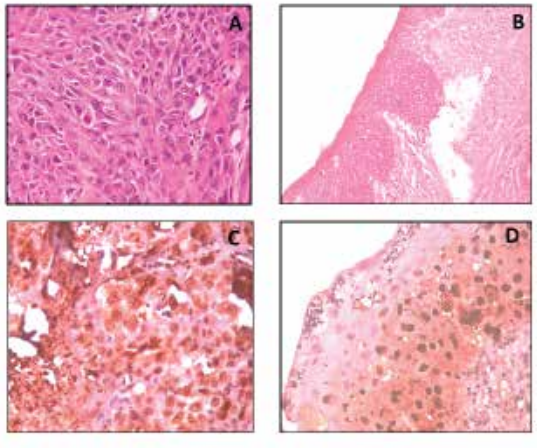

Figure 1: A. H and E stain grade III Oral Squamous Cell Carcinoma, B. H and E stain stratified squamous epithelium with marked dysplasia, C. p16 positive oral squamous cell carcinoma grade III showing diffuse marked nuclear and cytoplasmic staining, D. p16 positive moderately dysplastic stratified squamous epithelium with diffuse marked nuclear and cytoplasmic staining.

Out of 50 cases of OPL, 3 (6%) cases were positive for HPV. No significant association was observed between HPV positivity and other variables including age, gender, site, risk factors, clinical and histological grade of tumor.

Out of 100 cases of OSCC, HPV was positive in 15 (15%) cases. Significant association was noted between HPV and risk factors where HPV was predominantly positive in patients with smoking and betel quid chewing (p=0.029). Significant association was also seen between HPV and grade of tumor where HPV showed higher positivity in poorly differentiated OSCC (p=0.001). No significant association of HPV-16 was seen with other variables (Table 2) (Figure 2).

Table 2: Correlation of HPV with clinic-pathologic parameters in OSCC.

Parameters	HPV		Total	Chi sq *p*-value
	Negative (85%)	Positive (15%)		
Age Groups				
21-30years	6(66.7%)	3(33.3%)	9	0.442~
31-40years	21(87.5%)	3(12.5%)	24	
41-50years	30(88.2%)	4(11.8%)	34	
51-60years	20(90.9%)	2(09.1%)	22	
61-70years	6(75%)	2(25%)	8	
71-80years	2(66.7%)	1(33.3%)	3	
Gender				
Male	61(82.4%)	13(17.6%)	74	0.342
Female	24(92.3%)	2(07.7%)	26	
Risk Factors				
No Habits	8(88.9%)	1(11.1%)	9	0.029*~
Tobacco Smoking	12(75%)	4(25%)	16	
BQ /Tobacco chewing	44(95.7%)	2(04.3%)	46	
Smoking with BQ/Tobacco chewing	21(72.4%)	8(27.6%)	29	
Site				
Cheek	42(84%)	8(16%)	50	0.510~
Tongue	22(91.7%)	2(08.3%)	24	
Lip	5(71.4%)	2(28.6%)	7	
Alveolus	8(88.9%)	1(11.1%)	9	
Palate	4(100%)	0(0%)	4	
Floor	4(66.7%)	2(33.3%)	6	
Stage				
Stage I	4(66.7%)	2(33.3%)	6	
Stage II	18(90%)	2(10%)	20	

*Significant at 0.05, **Significant at 0.01, ~Chi-sq Test P-value with More than 20% Cells Proportion Test: Chi-square for association Fisher Exact Test.

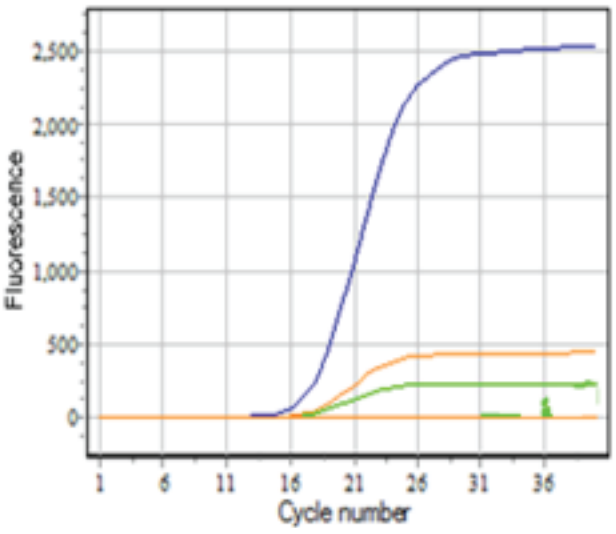

Figure 2: Crossing of fluorescence curve with the threshold line showing human DNA as Internal Control (amplification marker) detected on the Joe/HEX/Yellow channel;Positive HPV 16 Control on the FAM/Green channel; Positive HPV 18 Control on Rox/Orange channel.

OPL showed HPV DNA presence in dysplastic epithelial cells, strongly linked to p16 overexpression in 3 of 3 (100%) cases. However, 4 out of 7, of p16 positive cases did not show HPV DNA on Real time-PCR. (p = 0.001) (Table 3). Highly significant co expression of HPV with p16 was observed in all 15 (100%) HPV positive OSCC cases (p = 0.001) (Table 3).

Table 3: Correlation between p-16 and HPV in OPL and OSCC.

	OPL			*p*-value	OSCC			*p*-value
P16	HPV +ve	HPV -ve	Total	*<0.001	HPV +ve	HPV -ve	Total	*<0.001
+ve	3	4	7		15	3	18	
-ve	0	43	43		0	82	82	

*Chi square Significant at < 0.05

DISCUSSION

Oral squamous cell carcinoma was considered as a disease of elderly, but over the last few decades it has shown to be commonly affecting younger age group as well which is also observed in our study[17-19]. This shift to a decade earlier is likely to be related to the exposure by chemical carcinogens like tobacco and betel quid from an early age.

The present study reveals 91% of OSCC to be habitually exposed to chemical carcinogens with male predominance, where most of them were involved in the dual habits of smoking along with some form of tobacco chewing, which justifies double frequency of males being affected by OSCC[17,18,20] . We have detected more than half the cases (54%) in buccal cavity mucosa (cheek), which corresponds to the majority of the local studies[20-22].

While studies done in the West reported tongue as the commonest site of OSCC[23,24]. This can probably explained by the habit of local placement of tobacco, naswar and related quid material at the particular site. In recent decades, a number of studies have highlighted the molecular changes through various grades of dysplasia into the invasive malignant phenotype. Role of HPV and p-16 in OPL and OSCC is being extensively explored. Our study showed a similar trend of HPV prevalence (15%= OSCC and 6% = OPL) as noted by other studies[25-27].

Interestingly, a number of studies have shown higher prevalence of HPV in head and neck squamous cell carcinoma (HNSCC)[28-30]. This discrepancy can be sorted out as the high risk-HPV (HR-HPV) related tumors are more commonly detected in the oropharynx, posterior tongue and tonsillar pillars which are included in the head and neck classification but are outside the jurisdiction of WHO classification of oral cavity proper.

Considering the genotypes, we reaffirmed the fact with other studies that HR-HPV type 16 is more frequently the etiology of OSCC than type 18[14,27,29,31]. Although literature states that HPV is an independent risk factor for OSCC, Angel et al. reported tobacco associated carcinogens induces genetic alteration which might render HPV positive tumors less responsive to therapy. Our study observed significant correlation between HPV positive cases and chemical risk factors particularly in individuals with tobacco chewing habits, which justifies that other than tobacco smoking, chewable tobacco, which is much prevalent in our region, also has a significant association with HPV. We also noted a significant correlation of HPV positive cases with high-grade tumors, which similar to findings of Patil et al. and this might correlate with the virus overload, and an aggressive behavior of the tumor[32].

p16 tumor suppressor gene aberrations have been shown to be very common and one of the earliest molecular events in oral carcinogenesis[33,34]. In most HR-HPV unrelated oral cancers, inactivation of p16 by genetic and epigenetic mechanisms by chemical carcinogenesis leads to loss of immunoexpression. Comparable high frequencies of p16 inactivation, 86% in OPL and 82% in OSCC was seen in our study with a significant association with mild dysplasia and low grade tumors which is in accordance with other studies defining p16 loss as an early event[35,36].

Our study observed a significant co-expression of HPV with p16 in oral malignancies. Similarly, Patil et al. observed 86.6% of OSCC cases being p16 positive with HPV positivity in 87% of cases[32]. However; in our study, few cases, which showed p16 over expression, were noted to be HPV negative. Literature shows as many as 30% of p16 positive tumors have been reported to be HPV negative[13].The most feasible explanation in Rb inactivation caused by factors other than HPV infection needs to be explored. Pande et al. have also proposed a role of betel quid and tobacco in HPV unrelated tumors with p16 positivity[37].So, not only HPV but also chemical carcinogens might also be inactivating Rb gene. Alternatively, one might speculate that there might be unidentified genotypes of HPV that are not yet detectable by PCR. The other thought could be that tumors might have innate p16 over expression that develops independently from HPV.

CONCLUSION

In conclusion, HPV was found in substantial number of OSCC cases and few cases of OPL. Cancers of oropharynx and tonsils, which are common site for HR-HPV related tumors, were not included in the study and this could be one of the reasons of the low rates of HR-HPV prevalence in the present series. However, p16 was expressed in all HPV positive cancers justifying it as an adequate but not surrogate marker for HR-HPV in our population. To the best of our knowledge, only few studies are available from South Asian countries, which have observed a detailed study as ours in relation to p16, HPV and risk factors in premalignant and malignant lesions of oral cavity. One of the limitations was that we were unable to use more modern techniques other than DNA PCR for HPV-16/18, to identify mRNA for the E6/E7 viral transcripts for superior specificity due to difficult accessibility and financial constraints. However, our study is the first of its kind of study in Pakistani population, where we have highlighted the role of tobacco chewing, a major predominant risk factor, in p16 and HPV associated oral premalignant lesions and squamous cell carcinoma.

ACKNOWLEDGEMENTS

We thank Dow University of Health Sciences for allowing and supporting to conduct this research project.

CONFLICT OF INTEREST

There was no conflict of interest among the authors.

ETHICS APPROVAL

The study approval was sort from Dow University Ethics Review Committee (Reference number: Ph-D-13/ERB-64/DUHS-08).

PATIENTS CONSENT

Verbal and written informed consent was obtained from all patients.

AUTHORS CONTRIBUTION

SA and TM conceived the idea. SA collected the data and did bench work, further SA and AS wrote the manuscript. TM supervised the project and did critical review. All the authors proofread and approved the manuscript.

REFERENCES

1. Cheong SC, Vatanasapt P, Yi-Hsin Y, Zain RB, Kerr AR, Johnson NW. Oral cancer in South East Asia: Current status and future directions. Transl Res in Oral Oncology. 2017;2.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
3. Annual Cancer Registry Report-2017 of Shaukhat Khanum Memorial Cancer Hospital and Research Center, Pakistan [Internet]. 2018 [cited shaukhatkhanum.org.pk/wp-content/uploads/2018/08/acrr-2017.pdf].
4. Barnes L, Eveson J, Reichart P, Sidransky D. World Health Organization classification of tumours: pathology and genetics of head and neck tumours. Lyon: IARC Press; 2005.
5. Zygogianni AG, Kyrgias G, Karakitsos P, Psyrri A, Kouvaris J, Kelekis N, Kouloulias V. Oral squamous cell cancer: early detection and the role of alcohol and smoking. Head Neck Oncol. 2011;3(1):2.
6. Boffetta P, Hecht S, Gray N, Gupta P, Straif K. Smokeless tobacco and cancer. Lancet Oncol. 2008;9(7):667-75.
7. Su C-C, Yang H-F, Huang S-J, Lian I-B. Distinctive features of oral cancer in Changhua County: high incidence, buccal mucosa preponderance, and a close relation to betel quid chewing habit. J Formos Med Assoc. 2007;106(3):225-33.
8. Syrjänen S. Human papillomaviruses in head and neck carcinomas. N Engl J Med. 2007;356(19): 1993-5.
9. Gröbe A, Hanken H, Kluwe L, Schöllchen M, Tribius S, Pohlenz P, Clauditz T, Grob T, Simon R, Sauter G, Heiland M. Immunohistochemical analysis of p16 expression, HPV infection and its prognostic utility in oral squamous cell carcinoma. J Oral Pathol Med. 2013;42(9):676-81.
10. Muirhead DM, Hoffman HT, Robinson RA. Correlation of clinicopathological features with immunohistochemical expression of cell cycle regulatory proteins p16 and retinoblastoma: distinct association with keratinisation and differentiation in oral cavity squamous cell carcinoma. Clin Pathol. 2006;59(7):711-5.

11. Stephen JK, Divine G, Chen KM, Chitale D, Havard S, Worsham MJ. Significance of p16 in site-specific HPV positive and HPV negative head and neck squamous cell carcinoma. Cancer Clin Oncol. 2013;2(1):51.
12. Nemes JA, Deli L, Nemes Z, Márton IJ. Expression of p16INK4A, p53, and Rb proteins are independent from the presence of human papillomavirus genes in oral squamous cell carcinoma.Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006;102(3):344-52.
13. Lewis Jr JS, Thorstad WL, Chernock RD, Haughey BH, Yip JH, Zhang Q, El-Mofty SK. p16 positive oropharyngeal squamous cell carcinoma: an entity with a favorable prognosis regardless of tumor HPV status. AmJSurg Pathol. 2010;34(8).
14. Ndiaye C, Mena M, Alemany L, Arbyn M, Castellsagué X, Laporte L, Bosch FX, de Sanjosé S, Trottier H. HPV DNA, E6/E7 mRNA, and p16INK4a detection in head and neck cancers: a systematic review and meta-analysis. Lancet Oncol. 2014;15(12):1319-31.
15. Akhter M, Hossain S, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis.J Oral Maxillofac Pathol: JOMFP. 2011;15(2):168.
16. Sgaramella N, Coates PJ, Strindlund K, Loljung L, Colella G, Laurell G, Rossiello R, Muzio LL, Loizou C, Tartaro G, Olofsson K. Expression of p16 in squamous cell carcinoma of the mobile tongue is independent of HPV infection despite presence of the HPV-receptor syndecan-1. British J Cancer. 2015;113(2):321.
17. Hanif M, Zaidi P, Kamal S, Hameed A. Institution-based cancer incidence in a local population in Pakistan: nine year data analysis. APJCP. 2009;10(2):227-30.
18. Madni M, Jahromi A, Diikshit M, Bahaduri D, Aghamolaie T. Socio-demographic factors related to oral cancer. J Social Sci. 2010;6(2):141--5.
19. Gupta N, Gupta R, Acharya AK, Patthi B, Goud V, Reddy S, Garg A, Singla A. Changing Trends in oral cancer-a global scenario. Nepal JEpidemiol. 2016;6(4):613.
20. Shah I, Sefvan O, Luqman U, Ibrahim W, Mehmood S, Alamgir W. Clinical Stage of Oral Cancer patients at the time of initial diagnosis. J Ayub Med Coll Abbottabad. 2010;22(3):61-3.
21. Chen YJ, Chang JT, Liao CT, Wang HM, Yen TC, Chiu CC, Lu YC, Li HF, Cheng AJ. Head and neck cancer in the betel quid chewing area: recent advances in molecular carcinogenesis. Cancer Sci. 2008;99(8):1507-14.
22. Wahid A, Ahmad S, Sajjad M. Pattern of Carcinoma of oral cavity reporting at dental department of Ayub Medical College. J Ayub Med Coll Abottabad. 2005;17(1):65-6.
23. Patel SC, Carpenter WR, Tyree S, Couch ME, Weissler M, Hackman T, Hayes DN, Shores C, Chera BS. Increasing incidence of oral tongue squamous cell carcinoma in young white women, age 18 to 44 years. JClin Oncol. 2011;29(11):1488-94.
24. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009;45(4):309-16.
25. Isayeva T, Li Y, Maswahu D, Brandwein-Gensler M. Human papillomavirus in non-oropharyngeal head and neck cancers: a systematic literature review. Head Neck Pathol. 2012;6(1):104-20.
26. Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara Rey JM, García-García A. Genetic and molecular alterations associated with oral squamous cell cancer (Review). Oncology Rep. 2009;22(6):1277- 82.
27. Sritippho T, Pongsiriwet S, Lertprasertsuke N, Buddhachat K, Sastraruji T, Iamaroon A. p16-a possible surrogate marker for high-risk human papillomaviruses in oral cancer? APJCP. 2016;17(8): 4049-57.
28. Dufour X, Beby-Defaux A, Agius G, Lacau St Guily J. HPV and head and neck cancer.Eur Ann Otorhinolaryngol Head Neck Dis. 2012;129(1):26-31.
29. Hunt JL. An update on molecular diagnostics of squamous and salivary gland tumors of the head and neck.Arch Pathol Lab Med. 2011;135(5):602-9.
30. Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Cancer. 2010;11(1):9-22.
31. Schlecht NF, Brandwein-Gensler M, Nuovo GJ, Li M, Dunne A, Kawachi N, Smith RV, Burk RD, Prystowsky MB. A comparison of clinically utilized human papillomavirus detection methods in head and neck cancer. Mod Pathol. 2011;24(10):1295.
32. Patil S, Rao R, Amrutha N, Sanketh D. Analysis of human papilloma virus in oral squamous cell carcinoma using p16: An immunohistochemical study.J Int Soc Prev Community Dent. 2014;4(1):61.
33. Choi S, Myers J. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy.J Dent Res. 2008;87(1):14-32.
34. McGregor F, Muntoni A, Fleming J, Brown J, Felix DH, MacDonald DG, Parkinson EK, Harrison PR. Molecular changes associated with oral dysplasia progression and acquisition of immortality: potential for its reversal by 5-azacytidine. Cancer Res. 2002;62(16):4757-66.
35. Campo-Trapero J, Cano-Sánchez J, Palacios-Sánchez B, Sanchez-Gutierrez J, Gonzalez-Moles M, Bascones-Martinez A. Update on molecular pathology in oral cancer and precancer. Anti- cancer Res. 2008;28(2B):1197-205.
36. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma.J Clin Invest. 2012;122(6):1951-7.
37. Pande P, Mathur M, Shukla N, Ralhan R. pRb and p16 protein alterations in human oral tumorigenesis. Oral Oncol. 1998;34(5):396-403.