Original Article

Association of human papillomavirus and Epstein–Barr virus with squamous cell carcinoma of upper aerodigestive tract

ABSTRACT

Background: Cancers of the upper aero-digestive tract (UADT) are fifth most common cancer in the world with around 10,55,000 new cases and 7,25,000 deaths worldwide. Tobacco and alcohol act synergistically and are the two most important etiological factors responsible for about 75% of SCC. Studies have reported changing trends in the incidence of SCC showing an increasing shift in epidemiology attributed to the infection by viruses. The most commonly implicated viruses are the Human Papillomavirus (HPV) and Epstein-Barr virus (EBV).

Aim and Objective: To study association of Human Papilloma virus (HPV) and Epstein Barr virus (EBV) with 100 newly diagnosed cases of squamous cell carcinoma (SCC) of upper aerodigestive tract (UADT) and correlate its association with clinical parameters, histomorphological grade and staging using immunohistochemical markers p16, LMP1, p53, p63.

Materials and Methods: The study was conducted in the department of Pathology, Maulana Azad medical college and associated hospitals, New Delhi from September 2018-April 2019. Specimens was routinely processed. Immunohistochemistry (IHC) was done using p16, LMP1, p53, p63 monoclonal antibodies.

Statistical Analysis Used: SPSS 20 software. The quantitative analysis was done using Pearson chi-square test. Probability values < 0.05 was be considered statistically significant.

Results: HPV was present in 29% cases while EBV in 38% cases. Oral cavity was the most common site involved by both HPV and EBV. Co-infectivity was found in 4% cases. There was significant male predominance in both. HPV was more prevalent in age >45 years while EBV was equally distributed in <45 years and >45 years. Moderately differentiated squamous cell carcinoma was the commonest grade involved by both the viruses. A significant correlation was found between EBV and alcohol. p53 positivity had an inverse relationship with HPV positivity. P63 expression was higher in HPV and EBV positive cases.

Conclusion: In resource constraint settings, p16 and Latent membrane protein 1 can be used as surrogacy markers for Human Papilloma virus and Epstein Barr virus along with p53 and p63 for its association with histomorphological grade and stage.

Keywords: Epstein–Barr virus, human papillomavirus, latent membrane protein 1, P16, squamous cell carcinoma, upper aerodigestive tract

INTRODUCTION

Cancers of the upper aerodigestive tract (UADT) are fifth most common cancer in the world with around 1,055,000 new cases and 725,000 deaths worldwide. Squamous cell carcinoma (SCC) is the most common histological type (90%).[1] In India, oral cancer accounts for almost 30% of all cancers. The age-adjusted incidence is reported to be 20/100,000 population.[2]

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Tobacco and alcohol act synergistically and are the two most important etiological factors responsible for about 75% of SCC.[3] Studies have reported changing trends in the incidence of SCC showing an increasing trend, particularly in younger age group, especially in the absence of the above risk factors. This shift in epidemiology has been attributed to the infection by viruses. The most commonly implicated viruses are the human papillomavirus (HPV) and Epstein–Barr virus (EBV).[4]

HPVs are small nonenveloped icosahedral DNA viruses of Papovaviridae family. The virus comprises early and late genes that encode early proteins E1–E7and late proteins L1-L2.[5] HPV protein E6 binds to the p53 protein and inactivates it. This mechanism acts in synergy with the inactivation of the cell cycle regulator pRb by the HPV protein E7 which allows for repeated cell division.[6]

The involvement of HPV in oral and oropharyngeal carcinogenesis was first proposed by Syrjanen *et al*. in 1983.[7] By 2000-04, the proportion of HPV-positive cancers had risen to 72% which means that the rate of HPV-related oral cancer rose from 0.8 cases per 100,000 of the population in the 1980s to 2.6 cases per 100,000 in the 2000s; an increase of 225%.[8] In a study by Mofty *et al*. in 2007 in the USA in 235 cases of oropharyngeal carcinomas, in all age groups, 36% of tonsillar and 32% of base of tongue carcinomas were HPV related.[9]

EBV, also known as human herpesvirus 4 belongs to the Herpesviridae family, is an enveloped virus with double-stranded DNA genome. EBV encodes several viral proteins that have transforming potential, including EBV latent membrane proteins 1 and 2 (LMP1 and LMP2) and EBV nuclear antigens 2 and 3 (EBNA2 and EBNA3).[6]

The prevalence of EBV in oral samples varies widely between studies. Studies in South Asian countries found a high prevalence of EBV and suggested its etiological role in OSCC.[10]

A study conducted by Jalouli *et al*. showed that the highest HPV prevalence was seen in Sudan (65%), followed by India (45%) and the UK (45%) while EBV prevalence ranged from 22% in Yemen to 80% in the UK, with a statistically significant higher EBV prevalence in the industrialized countries compared to the developing countries.[11]

p16 is often deactivated in oral SCC. However, in HPV-related cervical cancer, oral cavity SCC and oropharyngeal SCC, p16 is markedly paradoxically overexpressed through negative feedback mechanism. However, when immunohistochemistry (IHC) and DNA studies were compared, it was seen only Grade III and Grade IV p16 positivity correlated with HPV positivity. In EBV-associated SCC, there is hypermethylation of p16 mediated by the LMP1-induced formation of a c-Jun/JunB heterodimer which in turn causes the activation of DNA methyltransferase. Hypermethylation is one of the most common mechanisms of p16 inactivation.[4] LMP1 deactivates p16 also by inducing the cytoplasmic accumulation of E2F4/5 and Ets2, which are nuclear proteins required for normal p16 activity causing its reduced immunohistochemical expression.[2]

HPV protein E6 binds to the p53 protein and inactivates it; there is reduced expression of p53 in HPV-positive cases.[6] EBV protein LMP1 does not inhibit p53 function through direct interaction. It induces expression of the cellular antiapoptotic genes bcl-2 and A20 and hence can block p53-mediated apoptosis resulting in reduced immunohistochemical expression of p53.[12]

p63 expression is inversely associated to grading of Oral SCC.[13] P63 is necessary for the activation of differentiation-dependent HPV late viral functions. Hence, immunohistochemically, there is over-expression of p63 in HPV-associated SCC.[14]

LMP1 is an EBV protein that acts as a functional homolog of tumor necrosis factor and mimics CD40 receptor pathway to prevent apoptosis of infected B-lymphocytes. It also induces the activation of several signaling pathways, including those of the NF-kappa-B family. Although LMP1 has no role in HPV infection, co-infectivity of HPV and EBV is rather on rise and is found in 15%–47% cases of nasopharyngeal carcinomas in various studies.[3,15,16]

MATERIALS AND METHODS

The study group included 100 cases of newly diagnosed upper aerodigestive tract SCC for which either small soft tissue biopsies or radical excision were conducted in the Department of Otorhinolaryngology, during the time period of October 2017–March 2019. Ethical clearance was received from Institutional Ethical Committee with reference number F.No.17/IEC/MAMC/2017/Path/04 dated 27/7/2017.

The group consisted of 87 males and 13 females, male: female ratio being 6.7:1 with age ranging between 15 and 87 years, median age 49.8 years. 81 cases were from oral cavity, 18 from oropharynx, and 1 from nasopharynx. History of tobacco and alcohol intake was taken whenever possible.

The samples were collected during surgery and sent to pathology department. Paraffin-embedded tissue blocks were made for the same. The h and e stained sections were graded according to the WHO criteria. The TNM staging was done during the primary diagnosis. IHC was done on sections on poly-L-lysine coated slides using p16 (PathnSitu, RTU), LMP1 (Dako, RTU), p53 (PathnSitu, RTU), and p63 (PathnSitu, RTU). The buffers used were PBS with pH 7.0 for staining and Citrate buffer with pH 6.0 for antigen retrieval. Diaminobenzidine was used as chromogen.

Interpretation of Immunohistochemistry

p16[82]: staining was taken as either cytoplasmic or nuclear.

- Negative
- 1+ 1-25% tumor cells positive
- 2+ 26-50% tumor cells positive
- 3+ 51-75% tumor cells positive
- 4+ >75% of tumor cells positive
- For surrogacy of HPV, only Grades III and IV were considered as positive[82].
- As complementary marker for EBV, all grades, I–IV was considered as positive.

Latent membrane protein 1

LMP1 expression was considered positive when more than 10% of tumor cells showed either cytoplasmic/membranous pattern of staining which had a granular pattern.

p53: p53 expression was considered positive when more than 10% of tumor cells showed nuclear pattern of staining

p63[140]: staining was taken as nuclear.

- <5% tumor cells positive
- 1+ 5-25% tumor cells positive
- 2+ 26-75% tumor cells positive
- 3+ >75% tumor cells positive

Statistical analysis was performed using Pearson's Chi-square test and with Fisher's exact test in SPSS software. *P* value < 0.5 was considered statistically significant.

RESULTS

The study comprised of 13 females with age ranging between 27 and 75 years and median age 40.5 years and 87 males with age ranging between 15 years to 87 years with median age 50 years [Figures 1 and 2]. The histomorphological grades included 27 well-differentiated SCC (WDSCC), 71 moderately differentiated SCC (MDSCC), and 2 poorly differentiated SCC (PDSCC) [Figures 3-5].

The TNM staging was calculated and comprised of 28 Stage I, 11 Stage II, 17 Stage III, 14 Stage Iva, and 2 Stage IVc. 75 out of 100 cases had a history of tobacco intake and 59 cases had a history of alcohol intake. 51 cases had history of both tobacco and alcohol intake.

75 cases were p16 positive, 38 LMP1 positive, 70 p53 positive, 90 p63 positive [Figures 6-9].

Of all the 100 cases, 29 cases were p16 Grade III and Grade IV positive and 38 cases were LMP1 positive, both with median age of 49.5 years and male: female ratio of 3.8:1 and 37:1, respectively.

In this study, HPV positivity was maximum in oral cavity, 18/81 (32%) followed by oropharynx, 3/18 (16.6%). The single case of nasopharyngeal SCC was negative for HPV. History of tobacco consumption was found in 21/29 (72.4%) p16-positive cases, maximum being in the age range of 41–50 years (6/21) while alcohol consumption was seen in 15/29 (51.7%) p16-positive cases, maximum being in age range

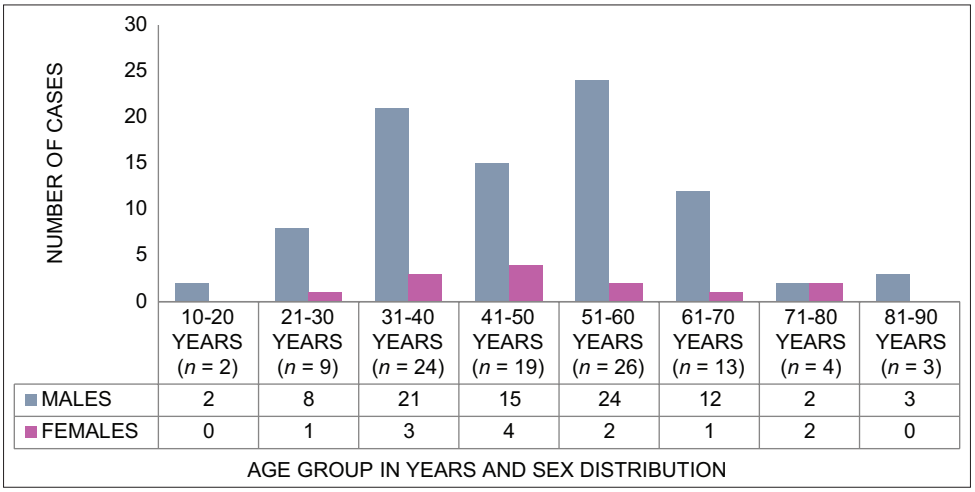

Figure 1: Age and sex distribution of cases

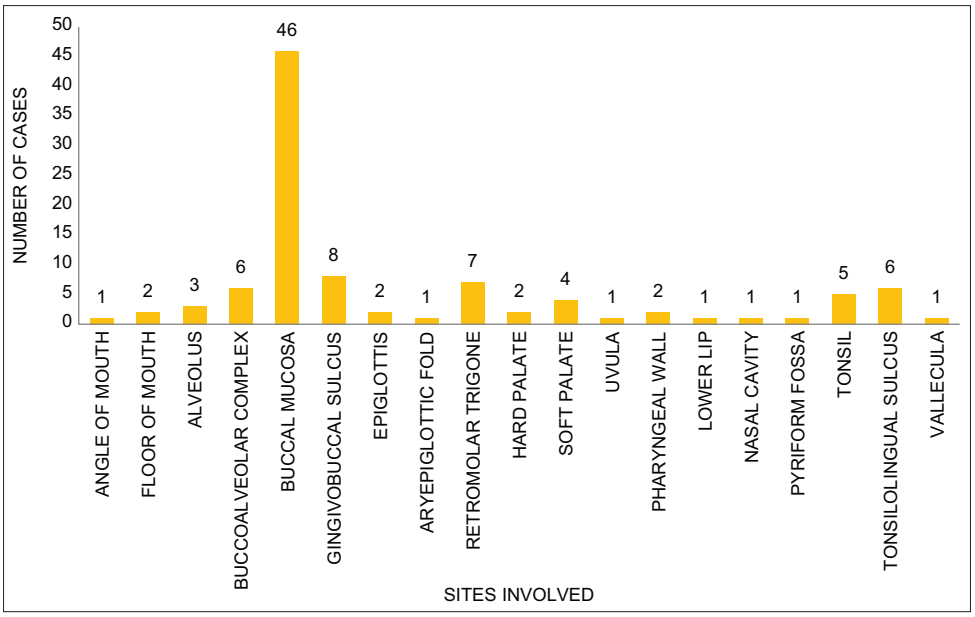

Figure 2: Distribution of cases as per site

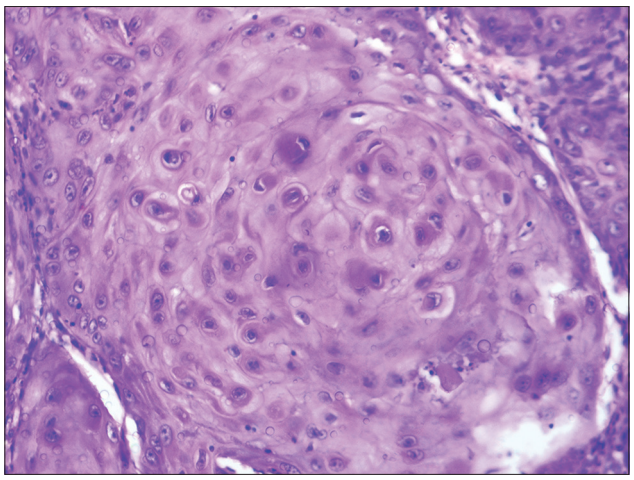

Figure 3: Microphotograph showing well-differentiated squamous cell carcinoma

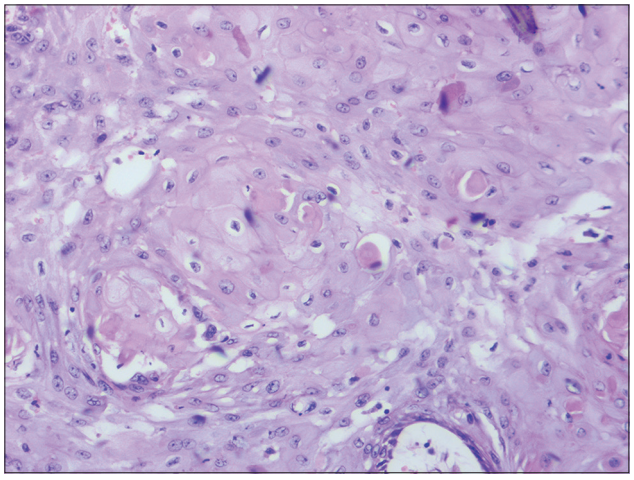

Figure 4: Microphotograph showing moderately differentiated squamous cell carcinoma (H and E, ×200)

41–50 years (6/15). Statistically, no significant correlation was found. 22/71 (30.9%) MDSCC cases, 6/27 (22.2%) WDSCC cases, and 1/2 (50%) PDSCC cases were p16 positive. No correlation was identified in this study based on grade of SCC except for those with Grade III p16 expression which had a *P* value of 0.000. Maximum p16 expression 9/28 (32.1%) was seen in Stage 1 SCC which comprised the maximum cases. The maximum percentage positivity, however, was seen in Stage 2 SCC, 6/11 (54.5%). A significant correlation of 0.005 was found. P53 was found to have significant inverse correlation with HPV. Out of the 70 p53-positive cases, only 15 (21.4%) cases were positive for HPV. Expression of p63 was found to be higher in HPV-positive SCC compared to HPV negative. 25/29 (86.2%) HPV-positive SCC expressed p63. The correlation was, however, statistically insignificant.

Maximum number of cases with EBV belonged to MDSCC followed by WDSCC followed by PDSCC. Percentage positivity was highest in WDSCC, 51.8% (14/27 cases) followed by PDSCC 50% (1/2 cases) followed by MDSCC, 32.4% (23/71 cases). Maximum EBV cases were detected in Stage 1 SCC, constituting 7/28 (32.1%) cases. Maximum percentage positivity of EBV was seen in Stage 4a, 7/14 (50%) cases. History of tobacco consumption in LMP1-positive cases was seen in 32/38 (84.2%) cases, maximum being in age range 51–60 years (9/32), and history of alcohol consumption was found in 28/38 (65.5%) cases, maximum in 51–60 years (9/28). A positive correlation with a *P* value of 0.013 was found between alcohol and LMP1 positivity. Out of the 70 p53-positive cases, 27 (38.5%) showed positivity for LMP1 as well. Nineteen cases were negative for both. However, no significant correlation was drawn between the

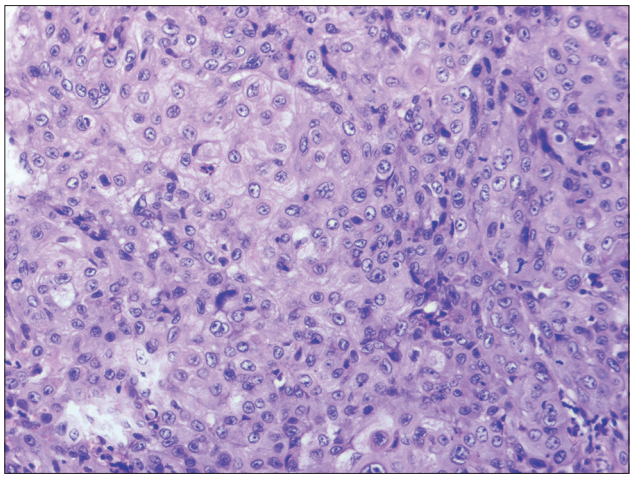

Figure 5: Microphotograph showing poorly differentiated squamous cell carcinoma (H and E, 200)

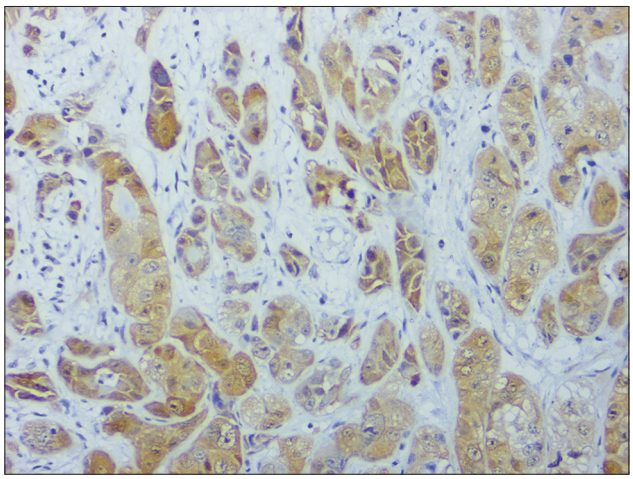

Figure 6: Microphotograph showing immunohistochemical marker p16 nuclear and cytoplasmic positivity (Grade III), using DAB as chromogen (×200)

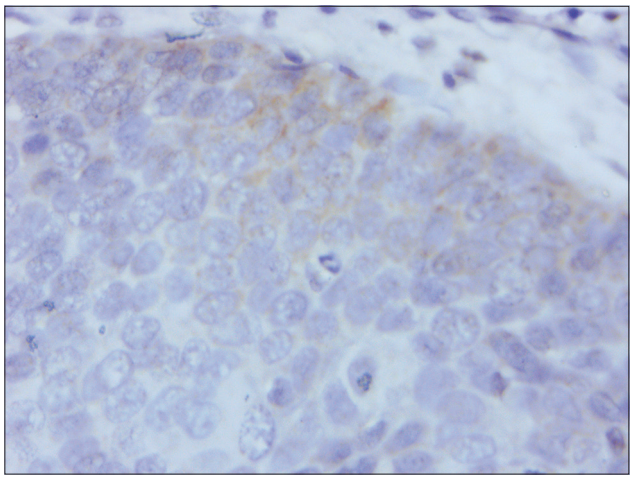

Figure 7: Microphotograph showing immunohistochemical marker latent membrane protein 1 cytoplasmic positivity, using DAB as chromogen (×600)

two. 37/38 also expressed p63. 24/37 (64.8%) were Grade III with a significant correlation of 0.001.

Co-infectivity was seen in 4/100 cases. Three were graded as MDSCC and one WDSCC case, all from oral cavity. This finding was also highly significant with a *P* value of 0.000.

DISCUSSION

Upper aerodigestive tract cancers are one of the most important causes of morbidity and mortality around the globe especially in developing countries like India. Various studies have described the role of viruses in carcinogenesis. HPV and EBV are two commonest viruses causing UADT SCC.

In this study, we correlated association of HPV and EBV with SCC of UADT and with demographic, clinical parameters as well as histomorphological grade and stage of UADT SCC.

Correlation with age

In this study, p16 expression had a positive correlation of 0.021 in patients >45 years of age and no correlation was found for EBV positivity.

Many authors noted that HPV-positive SCC was found more commonly in age <40 years.[17-19] While Elango *et al*.[20] and Liang *et al*.[21] found no significant difference between the young (≤45 years) and the old (>45 years).

Studies conducted on EBV by Broccolo *et al*.[22] and Higa *et al*.[10] showed that EBV positivity was found in age range of 42–90 years.

Correlation with sex

Various studies conducted had sex ratio between 4:1 and 4.7:1 for HPV positivity while this study had sex ratio of 3.7:1.[17,23-25]

The sex ratio for EBV positivity was 37:1 as there was only 1 female positive for LMP1 out of the 38 positive cases. This was in discordance with the study conducted by Higa *et al*. where the sex ratio ranged from 1.5:1 to 10:1 in different parts of Japan.[10]

Correlation with site

The most common primary site of origin of tumor in this study was the buccal mucosa comprising 46% of the cases. It has been seen that in India, buccal mucosa is indeed the most common site for SCC.[26] This may be because buccal mucosa being most exposed to chemicals carcinogens, infections, trauma is most vulnerable site to carcinogenesis.[27]

In the present study, HPV positivity was maximum in oral cavity, 18/81 (32%) followed by oropharynx, 3/18 (16.6%). This was in discordance with most studies like those by Maruyama

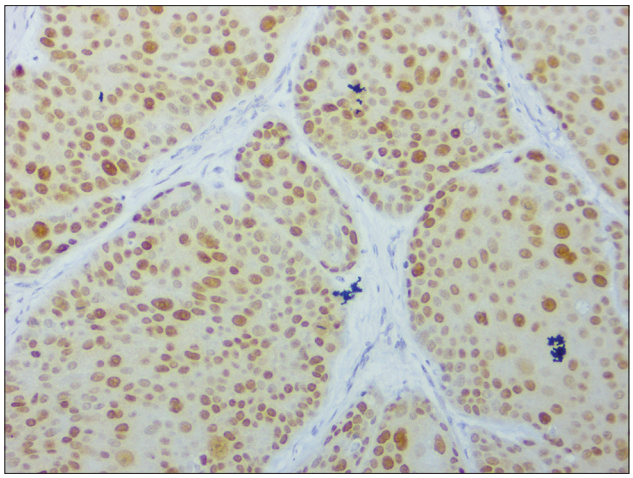

Figure 8: Microphotograph showing immunohistochemical marker p53 nuclear positivity, using DAB as chromogen (×200)

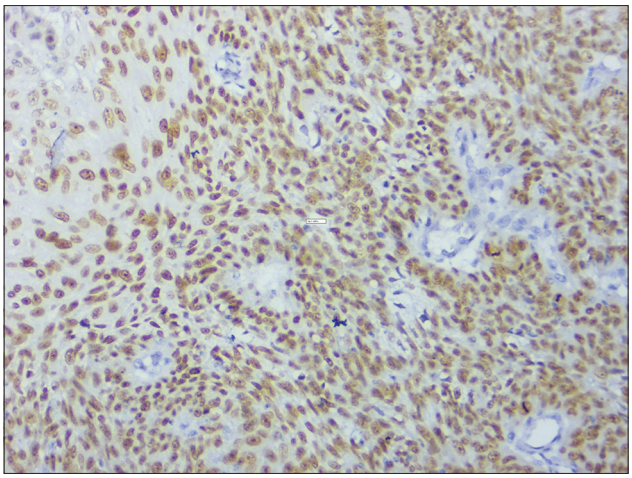

Figure 9: Microphotograph showing immunohistochemical marker p63 nuclear positivity Grade III, using DAB as chromogen (×200)

et al.,[25] Murthi *et al*.,[28] and Bahl *et al*.[29] where oropharynx was found to be the most common site for HPV-induced SCC.

LMP1 positivity was also observed highest in the oral cavity, 34/81 (41.9%) followed by oropharynx, 4/18 (22.2%). The single case of nasopharyngeal SCC was negative for LMP1. This finding was in concordance with the study by Broccolo *et al*.[22] who also found higher prevalence of EBV in oral cavity compared to oropharynx. Li *et al*. in their study found 82.5% of oral cancers specimens were EBV positive.[30]

Co-expression of p16 and LMP1 was seen in 4 cases, all were from oral cavity. Based on the expression of the markers on various sites involved, a positive correlation of 0.000 was found which is highly significant. Broccolo *et al*.[22] in a study in 2018 found co-infection of 10% of cases. Jalouli *et al*. found a co-infection of 21% (32/155) cases.

Correlation with clinical history

Tobacco and alcohol are preordained to cause UADT cancers. Although alcohol does not directly cause cancer, it fosters tobacco in doing so. History of tobacco and alcohol consumption was particularly highlighted in this study due to its inverse association with virus-induced carcinomas.[25]

Maruyama *et al*. conducted a study in 493 head-and-neck SCC, they noted that older patients, heavy smokers, and heavy drinkers were less likely to have HPV-positive cancers compared to the young, nonsmoker/light smoker and nondrinkers/light drinkers.[25] Other studies with similar findings were by Gillison *et al*.,[18] Smith *et al*.,[19] Karpathiou *et al*.,[31] and Murthi *et al*.[28]

In the present study, history of tobacco consumption in p16 and LMP1-positive cases was seen in 72.4% and 84.2%, respectively. This finding matched with that by Gruszka *et al*.[32] where a history of smoking was present in 77.7% of HPV-positive cases and in 76.2% of EBV-positive cases. The findings though were not significant statistically.

P16 and LMP1 expression in patients with a history of alcohol consumption was found in 50% and 65.5%, respectively. In this study, statistically, the correlation was significant (0.013) only for LMP1 and alcohol consumption. However, there was lack of literature showing any correlation between the two.

Histomorphological grade

According to the WHO, HPV prevalence is more commonly seen in nonkeratinizing carcinomas and EBV prevalence is seen more in undifferentiated or poorly differentiated carcinomas. Zhao *et al*.[33] found positive correlation between HPV infection and poor histological grade. Many authors though found higher prevalence of HPV in well-differentiated SCC.[20,34,35] In this study, 22/71 (30.9%) MDSCC cases, 6/27 (22.2%) WDSCC cases, and 1/2 (50%) PDSCC cases were p16 positive. No correlation was identified in this study based on grade of SCC except for Grade III p16 expression which had a $P = 0.000$.

Gonźalez-Moles *et al*.[36] showed a positive correlation between different grades of OSCC and EBV DNA positivity and also showed that percentage positivity of EBV DNA increases from well-differentiated OSCC to poorly differentiated OSCC. In this study, 23/71 (32.4%) MDSCC cases, 14/27 (51.8%) WDSCC cases, and 1/2 (50%) PDSCC cases were LMP1 positive. No significant correlation was found.

1/27 (3.7%) WDSCC cases and 3/71 (4.2%) MDSCC cases co-expressed p16 and LMP1 in this study. None of the 2 PDSCC cases, however, showed co-expression. This could be due to a

Table 1: Correlation of Epstein–Barr virus with P16

Authors	Al-Salam *et al*.[44] (*n*=95)	Zhao *et al*.[33] (*n*=60)	Kim *et al*.[42] (*n*=66)	Guenova *et al*.[46]	Present study (*n*=100)
Year	2013	2011	2006	1999	2017–2019
Correlation	Positive	Positive	Negative	No correlation	No correlation

smaller number of PDSCC cases in this study. The correlation was significant statistically with a $P = 0.021$.

Staging of squamous cell carcinoma

In this study, 72 cases were staged according to the AJCC guidelines. Maximum p16 expression 9/28 (32.1%) was seen in Stage 1 SCC which comprised the maximum cases. The maximum percentage positivity, however, was seen in Stage 2 SCC, 6/11 (54.5%). A significant correlation of 0.005 was found. This was in concordance with the studies by Agrawal *et al*.[37] and Mellin *et al*.[38] Maximum LMP1 expression was found in Stage 1 SCC, 9/28 (32.1%) while maximum percentage positivity was seen in Stage 4a, 7/14 (50%). This finding was, however, insignificant statistically and also could not be compared to any other studies due to paucity of literature.

Immunohistochemical markers

Virus-associated SCC is known to have an inverse relationship with p53 mutations. E6 of HPV leads to p53 degradation and hence its inhibition.[39] The same was noted in this study. Out of the 70 p53-positive cases, only 15 (21.4%) cases were positive for HPV (p16 Grades III and IV) while 55 (78.6%) were HPV negative (p16 negative/Grades I and II). 14/29 (48.2%) p16-positive cases were negative for p53 with a significant *P* value (0.010). Similar findings were noted by Gillison *et al*.,[18] Westra *et al*.,[40] and Maruyama *et al*.[21] in their studies.

LMP1 was positive in 27/70 (38.5%) p53-positive cases. However, no significant correlation was drawn between the two. Association of LMP1 and p53 was variable in different studies. Fries *et al*. noted in their study that LMP1-induced expression of p53 and antiapoptotic gene Bcl2 and A20 gene in epithelial cells prevents apoptosis in EBV-infected cells.[12] Al-Salam *et al*.[41] found positive correlation in their study on Hodgkin's Lymphoma, Kim *et al*.[42] showed no correlation between EBV and p53 while a study by Gracīa *et al*.[43] showed inverse relation between p53 and EBV.

Out of the 29 p16 Grades III and IV positive cases, 25/29 (86.2%) cases also expressed p63 suggesting that p63 was indeed expressed in HPV-positive cases. Since p63 was expressed in 90% cases in this study including in HPV negative cases, this finding was statistically insignificant.

Out of the 38 LMP1-positive cases, 37/38 also expressed p63. 24/37 (64.8%) were Grade III with a significant correlation of 0.001 proving p63 is highly expressed in EBV-positive SCC. Guo *et al*. in 2006 in China found in their study that p63 interacts with EBNA2 and hence imposes impact in LMP1 in nasopharyngeal carcinomas.[15]

One of the main mechanisms of EBV carcinogenesis in inactivation of pRb by LMP1 which further leads to overexpression of p16.[44] p16 hence may serve as a complementary marker for EBV irrespective of HPV status.[45] However, a lot of controversies are still present on the relationship of EBV with p16. Some studies correlating p16 expression with EBV is shown in Table 1.

Co-expression of p16 (all grades) and LMP1 was in 27 while co-expression of p16 Grades III and IV (for HPV surrogacy) and LMP1 was noted in 4 cases (4%), all in oral cavity. There was no significant correlation.

CONCLUSION

The study comprised of 100 newly diagnosed cases of SCC staged according to AJCC guidelines and confirmed on biopsy. The most common site involved was oral cavity. The most common grade was MDSCC. p53 expression was present in 70 cases, p63 expression in 90 cases, p16 expression in 75 cases, and LMP1 expression in 38 cases. P16 Grades III and IV, taken as surrogacy of HPV, however, were present in 29 cases. 15/29 HPV positivity detected by p16 expression (51.7%) was seen in ≤45 years of age while 14/29 (48.3%) was seen in >45 years of age. EBV positivity by LMP1 expression was equally distributed in ≤45 years and >45 years of age with 19/38 (50%) on each side. Male predominance was seen in both HPV and EBV-positive cases with male: female ratio of 3.8:1 and 37:1, respectively. Both HPV and EBV were significantly associated with site of the lesion, with maximum detection in the oral cavity. Co-infectivity of HPV and EBV was detected in 4% cases, all from oral cavity. Maximum number of cases with HPV and EBV belonged to MDSCC. The expression of p53 and p63 increased with increasing grade. Maximum HPV and EBV cases were detected in Stage 1 SCC, both constituting 7/28 (32.1%) cases, respectively. A significant correlation was found between EBV and alcohol. p53 was found to have significant inverse correlation with HPV. Expression of p63 was found to be higher in HPV-positive SCC compared to HPV negative. Higher grade of p63 expression was seen in EBV-positive cases. Therefore, p16 and p63 expression along with LMP1 is

recommended surrogacy markers for the prevalence of HPV and EBV in resource constraint settings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Gupta B, Kumar N, Johnson NW. A risk factor-based model for upper aerodigestive tract cancers in India: Predicting and validating the receiver operating characteristic curve. J Oral Pathol Med 2017;46:465-71.
2. Sankaranarayanan R, Masuyer E, Swaminathan R, Ferlay J, Whelan S. Head and neck cancer: A global perspective on epidemiology and prognosis. Anticancer Res 1998;18:4779-86.
3. Gandini S, Botteri E, Iodice S, Boniol M, Lowenfels AB, Maisonneuve P, *et al*. Tobacco smoking and cancer: A meta-analysis. Int J Cancer 2008;122:155-64.
4. Hammarstedt L, Lindquist D, Dahlstrand H, Romanitan M, Dahlgren LO, Joneberg J, *et al*. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. Int J Cancer 2006;119:2620-3.
5. Liang C, Marsit CJ, McClean MD, Nelson HH, Brock C, Christensen BC, *et al*. Biomarkers of HPV in head and neck squamous cell carcinoma. Cancer Res 2012;72:5004-13.
6. Gupta K, Metgud R. Evidences suggesting involvement of viruses in oral squamous cell carcinoma. Pathol Res Int 2013;2013:642496.
7. Syrjänen K, Syrjänen S, Lamberg M, Pyrhönen S, Nuutinen J. Morphological and immunohistochemical evidence suggesting human papillomavirus (HPV) involvement in oral squamous cell carcinogenesis. Int J Oral Surg 1983;12:418-24.
8. Metgud R, Astekar M, Verma M, Sharma A. Role of viruses in oral squamous cell carcinoma. Oncol Rev 2012;6:e21.
9. El-Mofty SK, Lu DW. Prevalence of human papillomavirus type 16 DNA in squamous cell carcinoma of the palatine tonsil, and not the oral cavity, in young patients: A distinct clinicopathologic and molecular disease entity. Am J Surg Pathol 2003;27:1463-70.
10. Higa M, Kinjo T, Kamiyama K, Chinen K, Iwamasa T, Arasaki A, *et al*. Epstein-Barr virus (EBV)-related oral squamous cell carcinoma in Okinawa, a subtropical island, in southern Japan-simultaneously infected with human papillomavirus (HPV). Oral Oncol 2003;39:405-14.
11. Jalouli J, Jalouli MM, Sapkota D, Ibrahim SO, Larsson PA, Sand L. Human papilloma virus, herpes simplex virus and epstein barr virus in oral squamous cell carcinoma from eight different countries. Int J Cancer Res Treatment 2011;32:571-80.
12. Fries K, Miller W, Raab-Traub N. Epstein-Barr virus latent membrane protein 1 blocks p53-mediated apoptosis through the induction of the A20 gene. J Virol 1996;70:8653-9.
13. Johnson NW. Oral cancer: A worldwide problem. FDI World 1997;6:19-21.
14. Kristen KM, Laimonis AL. p63 is necessary for the activation of human papillomavirus late viral functions upon epithelial differentiation. J Virol 2011;85:8863-9.
15. Guo C, Pan Z, Li D, Yun JP, Zheng M, Hu Z, *et al*. The expression of p63 is associated with the differential stage in nasopharyngeal carcinoma and EBV infection. J Transl Med 2006;4:23-31.
16. Yoshizaki T, Kondo S, Wakisaka N, Murono S, Endo K, Sugimoto H. Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. Cancer Lett 2013;337:1-7.
17. El-Mofty SK. Human papillomavirus (HPV) related carcinomas of the upper aerodigestive tract. Head Neck Pathol 2007;1:181-5.
18. Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. Semin Oncol 2004;31:744-54.
19. Smith EM, Ritchie JM, Summersgill KF, Klussmann JP, Lee JH, Wang D, *et al*. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer 2004;108:766-72.
20. Elango KJ, Suresh A, Erode EM, Subhadradevi L, Ravindran HK, Iyer SK, *et al*. Role of human papillomavirus in oral tongue squamous cell carcinoma. Asian Pac J Cancer Prev 2011;12:889-96.
21. Liang X. H, Lewis J, Foote R, Smith D, Kademani D. Prevalence and significance of human papillomavirus in oral tongue cancer: The Mayo clinic experience. J Oral Maxillofac Surg 2008;66:1875-80.
22. Broccolo F, Ciccarese G, Rossi A, Anselmi L, Drago F, Toniolo A. Human papillomavirus (HPV) and Epstein-Barr virus (EBV) in keratinizing versus non-keratinizing squamous cell carcinoma of the oropharynx. Infect Agents Cancer 2018;13:32.
23. Singhi AD, Westra WH. Comparison of human papillomavirus *in situ* hybridization and p16 immunohistochemistry in the detection of human papillomavirus associated head and neck cancer based on a prospective clinical experience. Cancer 2010;116:2166-73.
24. Hong A, Zhang X, Jones D, Veillard AS, Martin A, Lyons JG, *et al*. Relationships between p53 mutation, HPV status and outcome in oropharyngeal squamous cell carcinoma. Radiother Oncol 2016;118:342-9.
25. Maruyama H, Yasui T, Ishikawa-Fujiwara T, Morii E, Yamamoto Y, Yoshii T. Human papillomavirus and p53 mutations in head and neck squamous cell carcinoma among Japanese population. Cancer Sci 2014;105:409-17.
26. Chaturvedi P, Chocolatewala N. Role of human papilloma virus in the oral carcinogenesis: An Indian perspective. J Cancer Res Ther 2009;5:71-7.
27. Tsuchiya H, Tomita Y, Shirasawa H, Tanzawa H, Sato K, Simizu B. Detection of human papillomavirus in head and neck tumors with DNA hybridization and immunohistochemical analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1991;71:721-5.
28. Murthy V, Swain M, Teni T, Pawar S, Kalkar P, Patil A, *et al*. Human papillomavirus/p16 positive head and neck cancer in India: Prevalence, clinical impact, and influence of tobacco use. Indian J Cancer 2016;53:387-93.
29. Bahl A, Kumar P, Dar L, Mohanti B. K, Sharma A, Thakar A, *et al*. Prevalence and trends of human papillomavirus in oropharyngeal cancer in a predominantly North Indian population. Head Neck 2014;36:505-10.
30. Li C, Yen C, Lu M. Detection of EBV infection and gene expression in oral cancer from patients in Taiwan by microarray analysis. J Biomed Biotechnol 2009;2009:904589.
31. Karpathiou G, Monaya A, Forest F, Froudarakis M, Casteillo F, Dumollard JM, *et al*. p16 and p53 expression status in head and neck squamous cell carcinoma: A correlation with histological, histo-prognostic and clinical parameters. Pathology 2016;48:341-8.
32. Polz-Gruszka P, Morshed K, Stec A, Polz-Dacewicz M. Prevalence of Human papillomavirus (HPV) and Epstein-Barr virus (EBV) in oral and oropharyngeal squamous cell carcinoma in south-eastern Poland. Infect Agents Cancer 2015;10:37.
33. Zhao D, Xu Q, Chen X, Fan M. Human papillomavirus as an independent predictor in oral squamous cell cancer. Int J Oral Sci 2009;1:119-25.
34. Sgaramella N, Coates P, Strindlund K, Loljung L, Colella G, Laurell G, *et al*. Expression of p16 in squamous cell carcinoma of the mobile tongue is independent of HPV infection despite presence of the HPV-receptor syndecan-1. Br J Cancer 2015;113:321-6.
35. Md Ashraf J, Hosseini S, Monabati A, Valibeigi B, Khademi B, Abedi E, *et al*. The prevalence of human papilloma virus in squamous cell carcinoma of oral tongue Iran J Pathol 2017;12:144-9.
36. González-Moles M, Gutiérrez J, Ruiz I, Fernández J, Rodriguez M, Aneiros J. Epstein-Barr virus and oral squamous cell carcinoma in patients without HIV infection: Viral detection by polymerase chain reaction. Microbios 1998;96:23-31.

37. Agrawal G, Joshi P, Agrawal A. Role of HPV-16 in pathogenesis of oral epithelial dysplasia and oral squamous cell carcinoma and correlation of p16INK4A Expression in HPV-16 positive cases: An immunohistochemical study. Int Scholarly Res Notices Pathol 2013;2013:807095.
38. Mellin H, Friesland S, Lewensohn R, Dalianis T, Munck-Wikland E. Human papillomavirus (HPV) DNA in tonsillar cancer: Clinical correlates, risk of relapse, and survival. Int J Cancer 2000;89:300-4.
39. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell 1990;63:1129-36.
40. Westra WH, Taube JM, Poeta ML, Begum S, Sidransky D, Koch WM. Inverse relationship between human papillomavirus-16 infection and disruptive p53 gene mutations in squamous cell carcinoma of the head and neck. Clin Cancer Res 2008;14:366-9.
41. Martin HJ, Lee JM, Walls D, Hayward SD. Manipulation of the toll-like receptor 7 signaling pathway by Epstein-Barr virus. J Virol 2007;81:9748-58.
42. Kim LH, Peh SC, Poppema S. Expression of retinoblastoma protein and P16 proteins in classic Hodgkin lymphoma: Relationship with expression of p53 and presence of Epstein-Barr virus in the regulation of cell growth and death. Human Pathol 2006;37:92-100.
43. García JF, Camacho FI, Morente M, Fraga M, Montalbán C, Alvaro T, *et al*. Hodgkin and Reed-Sternberg cells harbor alterations in the major tumour suppressor pathways and cell-cycle checkpoints: Analyses using tissue microarrays. Blood 2003;10:681-9.
44. Al-Salam S, Awwad A, Sudhadevi M, Daoud S, Nagelkerke N, Castella A, *et al*. Epstein-Barr virus infection correlates with the expression of COX-2, p16(INK_4A) and p53 in classic Hodgkin lymphoma. Int J Clin Exp Pathol 2013;6:2765-77.
45. Jiang W, Chamberlain PD, Garden A, Kim B, Ma D, Lo EJ, *et al*. Prognostic value of p16 expression in Epstein-Barr virus-positive nasopharyngeal carcinomas. Head Neck 2016;38:1459-66.
46. Guenova M, Rassidakis GZ, Gorgoulis VG, Angelopoulou MK, Siakantaris MR, Kanavaros P, *et al*. P16INK4A is regularly expressed in Hodgkin's disease: Comparison with retinoblastoma, p53 and MDM2 protein status, and the presence of Epstein-Barr virus. Mod Pathol 1999;12:1062-71.