



CLINICOPATHOLOGICAL STUDY OF ORAL SQUAMOUS CELL CARCINOMAS WITH SPECIAL EMPHASIS ON E6, E7 ONCOPROTEIN EXPRESSION AND KI67 LABELLING INDEX

Pathology

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ABSTRACT

Introduction: Oral squamous cell carcinoma (OSCC) comprises of 95 per cent of all oral cancers and these represent approximately 5 per cent of cancers in men and 2 per cent in women. The expression of Ki67 is strongly associated with tumor cell proliferation and growth and is widely used in routine pathological investigation as a proliferation marker. The deregulated expression of the viral E6 and E7 oncoproteins has important role in neoplastic transformation by bypassing of cell cycle checkpoints. **Aims:** The aim of this study is to evaluate the clinicodemographic characteristics, prevalence of HPV infection status, and its correlation with proliferative activity of the tumor cells of OSCC in the Indian study population. **Material and Methods:** This study is Institution Based Observational Cross-Sectional Study conducted in the Department of Pathology, Medical College, Kolkata from January 2018-June 2019. 30 cases during the study period are included. **Result:** Clinical performance has been significantly affected by Tumor grade and E6, E7 expression and Ki67 index. A strong statistically significant association found in between E6/E7 positivity and Ki67 index; the latter being higher among E6/E7 strong positive cases. **Conclusion:** E6 and E7 alongside Ki67 can be used as predictive biomarkers of oral squamous cell carcinomas.

KEYWORDS

Oral Squamous Cell Carcinoma, E6 & E7 Expression, Ki67 Labeling Index, Proliferation, Prognosis.

INTRODUCTION:

Over 5 people in India die every hour and everyday because of oral cancer'. The International agency for research on cancer has predicted that India's incidence of cancer will increase from 1 million in 2012 to more than 1.7 million in 2035.²

High incidence of oral cancer in India is attributes to a number of etiological factors such as tobacco consumption habit among the patients either as smokeless tobacco or smoking, alcohol consumption, viral infections like HPV, poor oral hygiene.

High-risk human papillomavirus (HPV) is a causative agent for an increasing subset of oropharyngeal squamous cell carcinomas (OPSCCs). HPV subtypes 16 and 18 are the most-studied high-risk subtypes, but the majority of evidence in head and neck cancer has implicated HPV subtype 16 as an etiologic agent in more than 90% of HPV-related head and neck cancers.³

HPVs encode proteins that, creates an environment permissive for viral DNA replication. Central to these functions are the E6 and E7 genes.⁴ The E7 gene of the high-risk viruses binds the unphosphorylated form of the retinoblastoma protein, overriding the G1/S checkpoint of the cell cycle. The E6 gene of the high-risk HPVs binds p53 and targets p53 for ubiquitination. The combination of these events overrides cell cycle checkpoints and allows for viral DNA replication. An unfortunate but rare by-product of this is the deregulation of growth control in the infected cell and the development of cancer.

Ki67 antigen expression is a reliable and potent biomarker for the accurate, simple and prompt identification of the fraction of cells with proliferative potential in a tumor.⁵ Higher Ki67 expression is an indicator of tumor cells with higher proliferation and locally invasive potential and thus providing one of the best markers for the evaluation of biological aggressiveness of tumor.⁶ Ki67 expression has been accounted to provide a diagnostic and poor prognostic biomarker for OSCC patients.^{7,9}

MATERIAL AND METHOD:

Study design: Institution Based Observational Cross-Sectional Study.

Period of Study: January 2018-June 2019.

Study Population: Patients attending the OPD in Medical College.

Sample Size: 30 cases during the study period are included.

Study variables:

Sociodemographic factors,

Age, sex, high risk sexual practices, tobacco chewing, tobacco

smoking, alcohol consumption,

Clinical and Gross Examination findings

Tumor size, location, gross depth of invasion, resection margin status, lymph node number, size and location.

Histopathological findings

tumor grade, presence of necrosis, lympho-vascular invasion, margin status, pTNM stage.

IHC findings

Ki67 expression and Ki67 labelling index, presence E6/E7 oncoprotein of High risk HPV (mainly of HPV16)

IHC interpretation

The IRS (IMMUNOREACTIVE SCORE) system of SCORING was used for evaluation of E6 and E7 oncoprotein expression.

Ki67 Labelling index

Ki67 labelling index is determined according to standard guidelines.

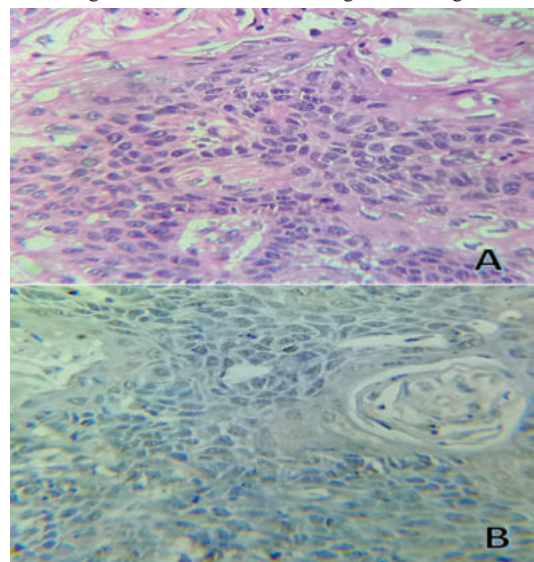


Figure 1.A: H&E Picture Showing Well-differentiated Squamous Cell Carcinoma (400x);

B: IHC STAINED SECTION WITH E6 SHOWING NEGATIVE STAINING

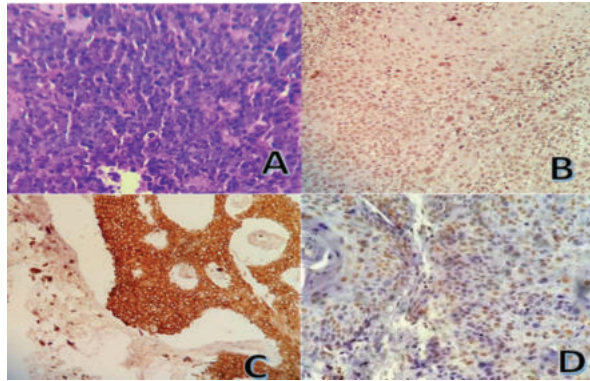


Figure 2.A: H&E Picture Showing Poorly Differentiated Squamous Cell Carcinoma (400x); B: IHC Stained Section Showing Strong Nuclear Positivity For E7; C: Strong Perinuclear And Cytoplasmic Positivity For E6; D: IHC Stained Section Showing Nuclear Positivity For Ki67

Statistical Methods:

The statistical software SPSS version 20 has been used for the analysis.

RESULT:

In our study, mean age of the cases, which were E6 strong positive and E7 strong positive, was 46 years (range 30years – 70years). These studies have also documented a relatively younger age in the HPV positive OSCCs.^{10,11,12}

Majority of total study population are male (60%).while majority of the patients with strong E6 positivity are female patients (60%). This finding contrasts with the findings of several other studies and review reports.^{13,14,15}

Only 20 percent of the patients were alcoholic, while 67 percent of the patients were tobacco chewers and 57 percent were smokers. There is a statistically significant association found between Alcoholism and 'Tumor Grade' ($p = 0.001$). Approx. 83 percent of alcoholic patients have Grade3 tumor while majority of the non-alcoholic group (63%) has Grade1 tumor. Interestingly, alcoholism is also found to be affecting the Ki67 Index significantly ($p = 0.018$). These findings support the fact that alcohol is possibly a worse risk factor in terms of prognosis than tobacco in cases of OSCC.^{16,17,18} There was no significant association among alcohol, Tobacco chewing, smoking with E6/E7 positivity, which is at par with findings of Applebaum et al.¹⁹ Tobacco Chewing and Smoking has no significant Association on tumor grade. These findings imply a synergistic effect of Alcohol and Tobacco on tumor biology in case of OSCC.²⁰

Majority of the patients (67%) were clinically Stable and functional but the Clinical performance has been significantly affected by Tumor grade and E6, E7 expression and Ki67 index with the debilitated ones showing stronger expression of E6/E7, higher Ki67 index and poorer grade. This finding is in contrast with the findings of Fakhry et al who concluded a better prognosis in HPV positive tumors.¹⁵

Among the oral cavity growths, majority (47%) were in the gingivobuccal sulcus among which 21% were 2nd Strongly positive for E6 and E7. Tongue was the most common site involved (37%) among which only 9 percent were strongly positive for E6 and E7. Among 'E6/E7Strong positive' cases 60 percent were situated at the gingivobuccal sulcus. Tumours of tongue and Gingivobuccal sulcus had larger size than tumors of angle of mouth and Retromolar trigone. Significant associations were found among the Tumor size and E6/E7 expression. Tumours of larger size were found to have strong expression of E6 and E7. These findings are in contrast to the findings of Heck et al.²¹ Tumor size also found to be significantly affecting the tumor grade.

In our Study, a significant association could not be concluded between Lymph node metastasis and E6/E7 expression; though in the "E6/E7Strong positive" cases a relatively advanced nodal stage (pN) is noted. This is in agreement with Fakhry et al. who also found nodal stage to be more advanced in HPV positive OSCC.¹⁵

All the E6/E7 strong positive cases were histologically keratinizing SCC which is in accordance with available literature.^{22,23} All the tumors which were E6/E7 strong positive (100%) showed a Depth of Invasion (DOI) of more than 5mm. Lymphovascular Invasion (LVI) was found in 34 % of cases overall while 80 % of E6/E7 strong positive cases has shown LVI. Statistical significance of E6/E7 positivity and LVI, PNI cannot be concluded from our study.

As far as Tumor Grade is concerned, Majority of cases (53%) were Well Differentiated SCC (Grade1). 27% cases were moderately differentiated (Grade2). 20 percent cases were poorly differentiated (Grade3). No statistical significance can be found between tumor stage and E6/E7 positivity.

Around 57% show positive staining with E6 implying a transcriptionally active HPV infection in the tumor cells in these cases. Among these, 17 percent tumors show a Strong positive (IRS 2 and IRS3) staining for E6. Rest of the cases (50%) show positive staining with E7 implying a transcriptionally active HPV infection in the tumor cells in these cases. Among these, 17 percent tumors show a Strong positive (IRS 2 and IRS3) staining for E7. Majority of the cases shown Ki67 index 2+ (83%) while 13percent cases show Ki67 index 3+ and only 1 case show Ki67 index 4+. A strong statistically significant association found in between E6/E7 positivity and Ki67 index; the latter being higher among E6/E7 strong positive cases.

DISCUSSION:

E6/E7 of HPV16 were strongly positive in 17 percent cases. The mean age of HPV positive cases is lower than overall population. A strong statistically significant association found in between E6/E7 positivity and Ki67 index; the latter being higher among E6/E7 strong positive cases.

Tumor grade is significantly affected by Alcohol, smoking, tobacco chewing, tumor size, Depth of invasion & E6/E7 positivity and in turn had significantly affected the clinical performance of the patients; Alcohol, Tobacco chewing, and smoking being related to worse tumor grade as is strong E6/E7 positivity.

Clinical performance has been significantly affected by Tumor grade and E6, E7 expression and Ki67 index with the debilitated ones (zubrod 3 and zubrod 4) showing stronger expression of E6/E7, higher Ki67 index and poorer grade. No statistical significance can be found between tumor stage and E6/E7 positivity.

Limitations

- Sample size was relatively small.
- Only biologic behavior of the tumor can be commented upon from a cross-sectional study. Prognosis can be anticipated only.

CONCLUSION:

In this Study, we conclude that the E6 and E7 oncoproteins of the high-risk Human Papilloma Virus significantly affects the tumor grade, tumor size as well as the proliferative activity of the tumor cells in Oral Squamous Cell Carcinomas. This indicates a possible aggressive biologic behavior of the HPV -positive OSCCs, particularly with transcriptionally active viral infection in the tumor cells. So, E6 and E7 alongside Ki67 can be used as predictive biomarkers of oral squamous cell carcinomas.

Poorer tumor grade is significantly associated with Alcoholism which is acting synergistically with Smoking and Tobacco chewing apart from HPV infection. A poorer clinical performance in the E6/E7 strong positive cases also implies a possible worse prognosis in HPV-positive OSCCs.

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