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A study on Ki-67 labeling index in oral squamous cell carcinoma

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Abstract
Background: Proliferative index is a measure of the number of cells in a tumour that are actually dividing (proliferating). The proliferative activity of any tissue or neoplasm can be determined by its growth rate using antibodies directed against specific antigens like KI-67.
Objective: This study is conducted to correlate cell proliferation in oral epithelial dysplasia (OED) and oral squamous cell carcinomas (OSCC) measured by anti-KI67 monoclonal antibody, thus determining its growth rate and to correlate KI-67 expression with histopathological grading and staging.
Method: Radical neck dissection specimen histopathologically diagnosed as squamous cell carcinoma with different grading and staging were followed by anti-KI 67 monoclonal antibody IHC study.
Result: The mean ki-67index in tumor center of well differentiated SCC (n=36), moderately differentiated SCC (n=21) and poorly differentiated SCC (n=3) were 29.6%, 30.4% and 26.8% respectively. The mean ki-67 index in proliferating margin of well differentiated SCC, moderately differentiated SCC and poorly differentiated SCC were 43.2%, 53.4% and 64.6% respectively.
Conclusion: The findings of the present study strongly argue for the value of highly expressed Ki-67 as an independent prognostic marker for OSCC.

Keywords: Ki-67, OSCC, squamous cell carcinoma, proliferative margin

Introduction

About 90% malignancies of oral cavity are due to squamous cell carcinoma [1]. In India and Asia, the chewing of betel liquid and pan is a major regional predisposing influence. This concoction, considered a delicacy by some, contains ingredients like areca nut, slaked lime, and tobacco, wrapped in a betel leaf; many of the ingredients of pan could give rise to potential carcinogens [2].

In the oropharynx, as many as 70% of Squamous cell carcinomas (SCCs), particularly involving the tonsils, base of tongue, and pharynx, harbor oncogenic variants of Human Papilloma Virus (HPV), particularly HPV-16. HPV-associated SCC of oropharynx has increased more than 2-fold over the last 2 decades. It is predicted that by the year 2020, the incidence of HPV-associated head and neck SCC will surpass that of cervical cancer, in part because the anatomic sites of origin (tonsillar crypts, base of tongue, and oropharynx) are not readily accessible or amenable to cytological screening (unlike the cervix) for premalignant lesions. Conversely, unlike the oropharynx, HPV-associated SCC of the oral cavity is relatively uncommon [2]. Survival is dependent on a number of factors including the specific etiology of SCC. The 5-year survival rate of 'classic' (smoking and alcohol-related) early-stage SCC is approximately 80%, while survival drops to 20% for late-stage disease. Patients with HPV-positive SCC have greater long-term survival than those with HPV-negative tumors. The dismal outlook of the classic SCC is due to several factors, including the fact that tumors are often diagnosed when the disease has already reached an advanced stage. Furthermore, the frequent development of multiple primary tumors markedly decreases survival. The rate of second primary tumors in these patients has been reported to be 3% to 7% per year, which is higher than for any other malignancy. The 5-year survival rate for the first primary tumor is considerably better than 50%, but in such individuals, second primary tumors are the most common cause of death. Therefore, the early detection of all premalignant lesions is critical for the long-term survival of this patients [2].

Proliferative index is a measure of the number of cells in a tumor that are actually dividing (proliferating). The proliferative activity of any tissue or neoplasm can be determined by its growth rate using antibodies directed against specific antigens like KI-67. KI-67 is a nuclear non-histone protein, encoded by a gene located on chromosome 10q25. It helps in regulation of cell cycle and is related to survival as well as prognosis of various malignant lesions.

The advantage of using KI-67 is that its expression occurs during almost all phases of the cell cycle, except in G0 phase and early G1 phase [5]. Since KI-67 is expressed in all proliferating cells and is of prognostic value in many cancers, it is a potential therapeutic target in cancer. Hence, the strategies that inactivate KI-67 protein are a promising anti-proliferative approach, and have potential application in treatment of cancers [3]. It has been claimed that a high cell-proliferation rate (which is measured by KI-67) in concordance with absence of TP-53 expression carries an excellent prognosis after radiation therapy, whereas tumors associated with expression of TP53 and having a low growth fraction with KI-67 <20% do not usually respond to radiation therapy [4].

This study is conducted to correlate cell proliferation in oral epithelial dysplasia (OED) and oral squamous cell carcinomas (OSCC) measured by anti-KI67 monoclonal antibody, thus determining its growth rate and to correlate KI-67 expression with histopathological staging. It provides an important objective criterion to determine the grade of oral squamous cell carcinoma. It can also help to determine the severity of oral epithelial dysplasia. Its expression is of special significance in diagnosis of moderately-differentiated or poorly-differentiated squamous cell carcinoma and moderate or poor epithelial cell dysplasia [3].

Materials and Methods

This study was conducted in histopathology department of Government Medical College, Bhavnagar from January to October 2019. Total 60 specimens of radical neck dissection histopathologically diagnosed as squamous cell carcinoma with different grading and staging were followed by anti-KI 67monoclonal antibody IHC study. Each case slide was analyzed for tumor center area and Proliferation margin. Findings were recorded and analyzed statistically.

Result

In the present study, 60 cases of neck dissection were studied to correlate KI-67 expression with grading and staging of squamous cell carcinoma. Grading and staging were given according to WHO TNM classification of carcinoma of the lip and oral cavity.

Table 1: KI-67 expression correlation with grading of squamous cell carcinoma.

Grading	No. of cases (60)	Tumor center		Proliferation margin	
		Mean (%)	SD	Mean (%)	SD
Well-differentiated	36	29.6	8.53	43.2	8.93
Moderately-differentiated	21	30.4	6.58	53.4	9.27
Poorly-differentiated	3	26.8	5.82	64.6	7.81

The mean ki-67index in tumor center of well differentiated SCC (n=36), moderately differentiated SCC (n=21) and poorly differentiated SCC (n=3) were 29.6%, 30.4% and 26.8% respectively. The mean ki-67 index in proliferating margin of well differentiated SCC, moderately differentiated SCC and poorly differentiated SCC were 43.2%, 53.4% and 64.6% respectively.

The mean ki-67 index also calculated in different stages of oral SCC. The mean ki-67index in tumor center was between 22.3 to 31.6% with SD of 5.9 to 7.3 in all stages of SCC. The mean ki-67 index in proliferating margin was between 41.1 to 56.6% with s SD of 6.8 to 10.7 in all stages of SCC. It can be concluded that Ki67 index have no significant prediction of tumor stage.

Table 2: KI-67 expression correlation with staging of squamous cell carcinoma.

Staging	No. of cases (60)	Tumor center		Proliferation margin	
		Mean (%)	SD	Mean (%)	SD
I	10	28.9	6.33	41.1	8.54
II	23	26.95	7.29	43	8.96
III	15	22.35	7.43	43.64	10.71
IVa	9	28.33	5.96	52.11	10.69
IVb	3	31.67	6.03	56.67	6.79
IVc	0	0	0	0	0

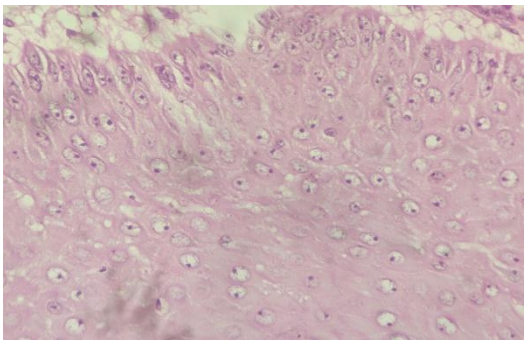

Fig 1: well differentiated SCC

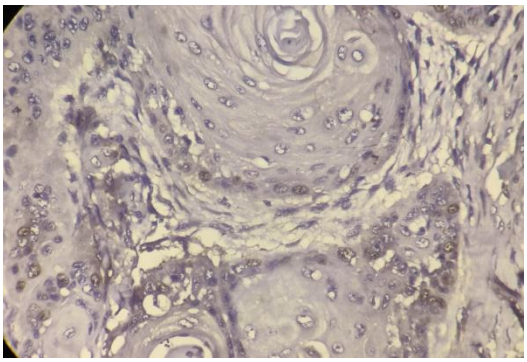

Fig 2: Ki-67, well differentiated SCC

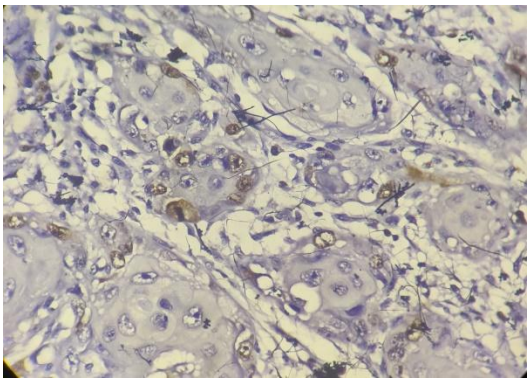

Fig 3: Ki-67, well differentiated SCC

Discussion

Oral squamous cell carcinoma (OSCC) is among the most frequently diagnosed cancer types worldwide. However, despite improved therapies, the 5-year survival rate has not changed. Overall, >50% of patients with OSCC demonstrate regional and distant metastases, which result in treatment failures and occasionally mortality within a year due to recurrent or metastatic disease [8, 9]. In order to improve the survival rate for OSCC, identification of an underlying molecular event differentiating patients at risk for progression at the premalignant stage is required [10]. Factors, including tumor size, lymph node metastasis, TNM type and differentiation, influence the prognosis of OSCC [11]. Proliferation marker protein Ki-67 (Ki-67), a nuclear and nucleolar protein, is expressed in proliferating cells from the G_1 to the M phase of the cell cycle, with the exception of the resting phase G_0 [5]. A sharp decrease in Ki-67 levels occurs in later phases of mitosis [6]. Additionally, Ki-67 has been shown to serve an important role in tumor genesis due to its positive association with tumor proliferation and invasion [7], providing a marker of tumor aggressiveness. Ki-67 is an indicator of cell proliferation and has been shown to be up regulated in numerous tumors [12, 13]. Tumor proliferative activity labeled by Ki-67 has been found to be associated with tumor aggression, which is specified by tumor grade and stage. Several studies have described these associations and identified Ki-67 as a prognostic factor [14-16]. The present study found up regulation of Ki-67 expression with tumor progression using normal epithelial mucosa, dysplasia and OSCC samples. In addition, Ki-67 expression also increased from mild to moderate to severe dysplasia. Maryam *et al.* [17] reported that Ki-67 expression was significantly related to histological grading, and was significantly lower in the low-grade group. Tumulari *et al.* [18] and Huang *et al.* [19] reported that well-differentiated tumors have the lowest mean Ki-67 immunostaining. Kurokawa *et al.* [20] reported that overexpression of Ki-67 at the deep tumor invasive front of OSCC is associated with histologic grade of malignancy. Watanabe *et al.* [21] reported that the expression of Ki-67 was significantly stronger in OSCC than normal oral mucosa. Fabricio LD Vieira *et al.* [22] observed that the comparison between the percentages of tumor cells positive for Ki-67 expression per microscopic field in the oral mucosa samples of the three groups (well-differentiated, moderately-differentiated and undifferentiated respectively) showed statistically significant differences ($p<0.05$). Tumors from the undifferentiated group showed a greater expression of protein Ki-67 compared to the tumors from other 2 groups. Pich *et al.* [23]. (2004) in a retrospective study with malignant lesions of the mouth cavity, salivary glands, pharynx, and larynx, observed that the proliferative activity investigated by different methods, including Ki-67 expression by immunohistochemistry, is clinically relevant and valid for proposing treatment and defining prognosis. Marinescu *et al.* [24] found positivity for Ki-67 in all poorly and moderately differentiated tumors regardless of tumor stage and only in 61.1% of the well-differentiated tumors. Buch *et al.* [25] observed that in case of tumor proper (TP), Ki-67 labeling index (LI) was lowest for well-differentiated SCC (LI=29.96%) and highest for moderately-differentiated SCC (LI=34.96%). A statistically significant increase was noted with grade of oral SCC, with highest mean Ki-67 LI being 66% in case of poorly-differentiated SCC. Study by Dissanayake *et al.* [26] stated that in oral SCC, more cells in the invasive tumor front are in the proliferative state as compared to the center of the tumors. Pity and Jalal [27] showed that highest levels of Ki-67 labeling index were seen in poorly-differentiated SCC.

In our study, the mean ki-67 index in proliferating margin of well differentiated SCC, moderately differentiated SCC and poorly differentiated SCC were 43.2%, 53.4% and 64.6% respectively. The mean ki-67 index also calculated in different stages of oral SCC. The mean ki-67index in tumor center was between 22.3 to 31.6% with SD of 5.9 to 7.3 in all stages of SCC.

Conclusion

The findings of the present study strongly argue for the value of highly expressed Ki-67 as an independent prognostic marker for OSCC. Considering the extensive heterogeneity of tumors, further researches need to enrich correlations of spatial expression of Ki67 with OSCC. Ki-67 can reliably detect the proliferative potential of cells at the invasive margin of a tumor.

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