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Original Research Article

# Ki-67 Index in Oral Squamous Cell Carcinoma: Two Year Study at a Tertiary Care Centre of Kashmir

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**Abstract**

**Introduction:** Squamous cell carcinoma of the oral cavity is among ten most common cancers in the world, and accounts for almost 3-5% of all the malignancies. Ki-67 is non-histone nuclear protein, that helps in regulating the cell cycle. It is related to survival as well as prognosis of various neoplastic lesions. The advantage of using Ki-67 is that its expression occurs in almost all phases of the cell cycle, except in G0 phase and early G1 phase.

**Material and Methods:** The study was conducted in the department of pathology sher-i-Kashmir institute of medical sciences Srinagar Kashmir from January 2019 to december 2020. The case history of 60 patients histo-pathologically diagnosed as squamous cell carcinoma with different grading and staging was transcribed from patient's individual clinical records followed by anti-Ki 67 monoclonal antibody IHC study. Each case slide was analyzed for ki-67 index at Proliferation margin. Paraffin sections of formalin-fixed tissues were used for both histological and immunohistochemical evaluation.

**Results:** Correlation of Ki-67 index was done with grade and stage of squamous cell carcinoma. The mean ki-67index in well differentiated OSCC (n=38), moderately differentiated OSCC (n=20) and poorly differentiated SCC (n=2) was 41.2%, 55.4% and 65.6% respectively, with standard deviation of 8.9- 9.27 and a significant P-value of 0.012.

**Conclusion:** It can be concluded that Ki-67 index has no significant prediction in tumor stage, while as ki-67 index was found to have direct prediction in grade of the tumor, I,e: higher the grade of tumor, higher was ki-67 index and vice versa.

**Keywords:** Ki-67 index, Squamous cell carcinoma, Tumor grade.



## Introduction

Squamous cell carcinoma of the oral cavity is among ten most common cancers in the world, and accounts for almost 3-5% of all the malignancies. Oral squamous cell carcinoma (OSCC) corresponds to 95% of all head and neck cancers and is associated with high morbidity and mortality [1]. Its high frequency in Central and South East

Asian countries (India, Bangladesh, Sri Lanka, Thailand, Indonesia, Pakistan) is attributed to the chewing of betel liquid and pan [2]. This concoction, contains ingredients like areca nut, slaked lime, and tobacco, wrapped in a betel leaf; that could give rise to potential carcinogens [3].

Histopathological grading of squamous cell carcinoma (SCC) was first introduced in 1920 by Broder's. According to his system he classified OSCC as well-differentiated squamous-cell carcinoma (WDSCC), moderately differentiated squamous-cell carcinoma (MDSCC) and poorly differentiated squamous-cell carcinoma (PDSCC) [4]. Measure of number of cells in a tumor that are actually dividing (proliferating) is termed as Proliferative index. The proliferative index of any tissue or tumor can be assesed by its growth rate using antibodies that are directed against specific antigens like Ki-67.

Ki-67 is non-histone nuclear protein, that helps in regulating the cell cycle. It is related to survival as well as prognosis of various neoplastic lesions. The advantage of using Ki-67 is that its expression occurs in almost all phases of the cell cycle, except in G0 phase and early G1 phase [5]. As it is expressed in all proliferating cells and is of prognostic value in many cancers, it is a potential therapeutic target in carcinomas. Hence, the strategies that inactivate Ki-67 protein are a promising anti-proliferative approach, and have potential application in treatment of cancers [6]. Ki-67 is highly abundant and the epitope recognized by the Ki-67 monoclonal antibody (FKELF) is naturally amplified, being present on nine of the 16 Ki-67 repeats that comprise much of the polypeptide [7]. These features make Ki-67 one of the best markers to assess cell proliferation and its use as a reagent to aid in determining a patient prognosis [8]. 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 Ki-67 fraction <20% do not usually respond to radiation therapy [9].

Although Ki-67 has been reported to provide a diagnostic marker for neck metastasis in head and neck carcinomas, its role in OSCC has not been fully clarified. The present study aimed to evaluate the association between Ki-67 expression with different histological grades and stage of oral squamous cell carcinomas to further assess its diagnostic value.

## Material and Methods

The study was conducted in the department of pathology sher-i- Kashmir institute of medical sciences Srinagar Kashmir from January 2019 to december 2020. The study material included both tongue and other intra-oral SCCs. The case history of 60 patients histo-pathologically diagnosed as squamous cell carcinoma with different grading and staging was transcribed from patient's individual clinical records followed by anti-Ki 67 monoclonal antibody IHC study. Each case slide was analyzed for ki-67 index at Proliferation margin. Paraffin sections of formalin-fixed tissues were used for both histological and immunohistochemical evaluation. Hematoxylin and eosin stained sections of 4 µ were used for routine histological examination.

**Immunohistochemical** (IHC) for Ki-67 index was performed using DAKO-LSAB-2HRP detection system. For IHC 4 µm thick sections were cut using rotatory microtome. Sections were placed on pre-coated slides and incubated for 1 h at 60°C. For antigen retrieval, the sections were placed in a 1mM citrate buffer (pH 6) and microwave was used with cycles of high, medium, and low each lasting 5 min subsequently bringing to room temperature. The endogenous peroxidase activity was blocked by using 3% hydrogen peroxide for 10 min followed by washing in 0.05mM Tris-buffered saline (TBS). The sections were incubated with diluted mouse

monoclonal antibodies against Ki-67 (MIB-1prediluted Dako, Japan) as primary antibodies for 1 h at 37°C. Subsequently, the sections were incubated with a secondary antibody conjugated with peroxidase-labelled dextran polymers for 30 min at room temperature. After rinsing with TBS, sections were treated with 3, 3'-diaminobenzidine solution containing 0.001% hydrogen peroxide to visualize the reaction products. Sections were counterstained with hematoxylin for 3 min. Findings were recorded and analyzed statistically.

**Statistical analysis:**

IHC slides were examined for positive staining by light microscopy. The mean percentage of positively stained cells was estimated by counting 300 cells per area in a minimum of five different fields representative of the histology. The nuclear expression of Ki-67 was counted using a microscope at × 400. Statistical analysis was considered significant where 'P'-value was < 0.05.

**Results**

All the 60 cases were primary oral squamous cell carcinoma. Out of 60 cases 40 (66.7%) patients were males and 20 (33.3%) patients were females. 50 (83.3%) patients were more than 50 years of age whereas rest of the 10 (16.7%) patients belonged to age group of 50 years or less. The mean age of presentation being 59 yrs. 60% of the patients had associated history of tobacco use. Tongue was the most common site of involvement

contributing to 33.3% of the oral SCC. (Table-1)

**Table 1: Characteristics of the study group.**

| Features | Number of patients | Percentage (%) |
|---|---|---|
| **Age at presentation (years)** | | |
| ≤50 | 10 | 16.7 |
| >50 | 50 | 83.3 |
| **Gender** | | |
| Male | 40 | 66.7 |
| Female | 20 | 33.3 |
| **Tobacco (smoking/chewing** | | |
| Yes | 36 | 60 |
| No | 24 | 40 |
| **Anatomical site** | | |
| Tongue | 20 | 66.7 |
| Other oral site | 40 | 33.3 |
| **T stage** | | |
| T1+T2 | 19 | 31.6 |
| T3+T4 | 41 | 68.4 |
| **Nodes** | | |
| N0 | 38 | 63.3 |
| Nodes clinically/pathologically present | 22 | 36.7 |

Tumor differentiation was assessed which showed that 38 cases (63.3%) were well differentiated, 20 cases (33.3%) were moderately differentiated and 2 cases (3.4%) of the were poorly differentiated.

Grading and staging was done according to WHO TNM classification system for carcinoma of the lip and oral cavity. Correlation of Ki-67 index was done with grade and stage of squamous cell carcinoma.

The mean ki-67index in well differentiated OSCC (n=38), moderately differentiated OSCC (n=20) and poorly differentiated SCC (n=2) was 41.2%, 55.4% and 65.6% respectively (fig1 & 2), with standard deviation of 8.9- 9.27 and a significant P-value of 0.012 (Table-2)

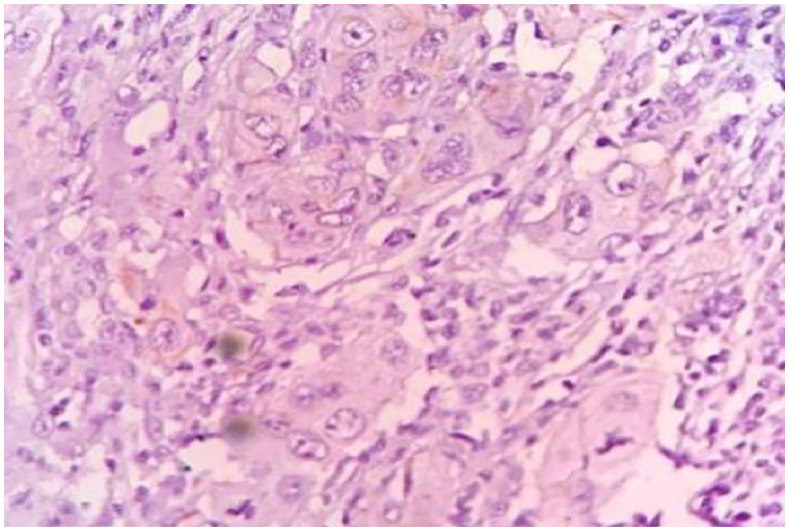

**Figure 1: Photomicrograph showing moderately differentiated Squamous cell carcinoma (H&E)**

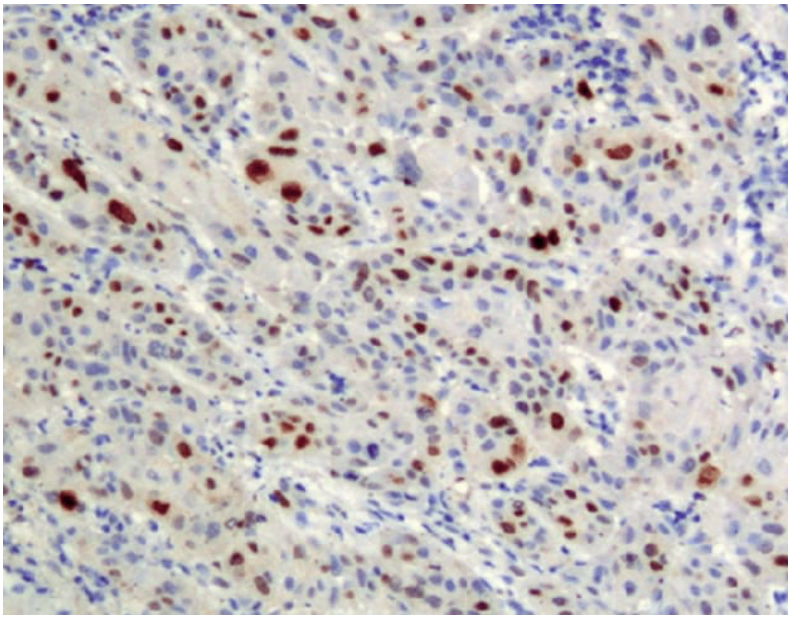

**Figure 2: Photomicrograph showing ki-67 positivity in nuclei of proliferating tumor cells in moderately differentiated squamous cell carcinoma.**

**Table 2: Correlation of Ki- 67 expression with grade of squamous cell carcinoma**

| Grading | No. of cases (60) | Ki-67 index Mean (%) | SD (standard deviation) | P-value |
|---|---|---|---|---|
| Well-differentiated | 38 | 41.2 | 9.0 | 0.012 |
| Moderately differentiated | 20 | 55.4 | 9.27 | 0.012 |
| Poorly differentiated | 2 | 65.6 | 8.9 | 0.012 |

The mean ki-67 index was also calculated in different stages and was found to be between 41.1 to 56.6% in all stages of OSCC (Table-3).

**Table 3: Correlation of Ki-67 index with staging of squamous cell carcinoma**

| Stage | No. of cases (60) | Mean ki-67 index (%) | SD (standard deviation) | p-value |
|---|---|---|---|---|
| I | 10 | 41.1 | 8.6 | 0.314 |
| II | 23 | 43 | 8.8 | 0.314 |
| III | 15 | 43.64 | 10.70 | 0.512 |
| IV a | 9 | 50.11 | 10.70 | 0.512 |
| IV b | 3 | 56.67 | 6.8 | 0.224 |

## Discussion

The transition of the normal epithelium of oral cavity to dysplasia and subsequently to malignancy is indicated by increase in cell proliferation [10]. Proliferation markers play a significant role in the biological behaviour of tumors. Various proliferation markers have been discovered that enable the detection of the proliferative state of the epithelium and have been thought to be of prognostic significance [11]. The Ki-67 monoclonal antibody is one such proliferation marker that is commonly used. It is reactive against the nuclear antigen Ki-67 that is expressed during G1, S, G2, and M phases cell cycle but is not found during G0 [12]. A number of diagnostic applications for Ki-67 protein have been described, where Ki-67 was significantly more highly expressed in malignant than in normal tissues. The significance of Ki-67 protein as a prognostic marker has been widely studied. Ki-67 represents an additional predictor of survival in breast cancer, cervical, uterine cancer, non-Hodgkin's lymphoma and large bowel cancer [13]. Expression of Ki67 can be used as a prognostic biomarker in Colorectal cancer (CRC) (14). Furthermore, it has been considered to be one of the best predictors of survival and recurrence. Ki-67 has been shown to serve an important role in tumor genesis due to its positive association with tumor proliferation and invasion 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 [15]. 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 [16].

Maryam *et al.* reported that Ki-67 expression was significantly related to histological grading, and was significantly lower in the low-grade group [17]. Huang *et al* reported that well-differentiated tumors have the lowest mean Ki-67 immunostaining [18]. Kurokawa *et al* showd overexpression of Ki-67 at the deep tumor invasive front of OSCC is associated with histologic grade of malignancy [19]. Pich *et al* 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. [20]. Pity and Jalal showed that highest levels of Ki-67 labeling index were seen in poorly-differentiated SCC [21]. Rakheja Mahima et al in 105 cases showed closely similar results. A highly significant correlation was found between the expression of Ki-67 and grades of OSCC ($p<0.001$) on statistical analysis. Hence this clearly told that in OSCC, the rate of cell proliferation increase with the decrease in the degree of tumour differentiation [22].

In our study, the immunoexpression of Ki-67 was seen in all 60 cases of OSCC. The relationship of Ki-67 expression was

statistically significant when compared with histological grading (p value=0. 012).

## Conclusion:

It can be concluded that Ki-67 index has no significant prediction in tumor stage, while as it was found to have direct prediction in grade of the tumor, I,e: higher the grade of tumor, higher was ki-67 index and vice versa. we conclude that the expression of Ki ‑67 increased progressively with the grade of OSCC.

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