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# Role of P53 and KI67 in oral squamous cell carcinoma: An immunohistochemical study

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**Abstract: Background:** Oral squamous cell carcinoma is a carcinoma with squamous differentiation arising from the mucosal epithelium. The pathogenesis of oral cancers is multifactorial. P53, guardian of the genome regulates cell cycle progression, DNA repair, cellular senescence and apoptosis. Ki-67 is a cell cycle associated nuclear protein used as a proliferation marker to measure the growth fraction of cells in human tumours.
**Aims and Objectives:** To compare demographic factors like age, sex and predisposing factors. To evaluate grade of differentiation, expression of p53, Ki67 and their comparison in various grades of differentiation in oral squamous cell carcinomas.
**Materials and Methods:** This study comprises of 35 cases of oral squamous cell carcinoma After processing of representative tissue block, H&E and IHC stain with Ki-67 and p53 immunomarkers were carried out.
**Results:** Out of 35 cases of oral squamous cell carcinoma,20(57%) were well differentiated, 14(40%) moderately differentiated, 1(3%) was poorly differentiated. The age range was 26-85 years. Sex ratio was 2:1. The most common risk factor for development of cancer was found to be smoking (57%) followed by betel quid chewing (43%). The most common clinical presentation was non healing ulcer (80%). Sensitivity of Ki67 and P53 is 100% and 63% respectively.
**Conclusion:** P53 positivity was demonstrated in majority of cases indicating that it is most common genetic mutations in oral cancers. The present study showed an inverse correlation between the degree of tumour differentiation and the rate of cell proliferation obtained by the expression of Ki-67.

**Keywords:** OSCC-Oral squamous cell carcinoma.

## 1. Introduction

Squamous cell carcinomas accounts for 95% of cancers of the head and neck. Head and neck cancer includes tumours of the oral cavity, pharynx, larynx, nasal cavities, thyroid and salivary glands [1]. Oral squamous cell carcinoma is a carcinoma with squamous differentiation arising from the mucosal epithelium [2]. Worldwide, oral cancer is the sixth most prevalent cancer, ranking eighth in developed countries and third in the developing world [3]. Squamous cell carcinoma account for more than 90% of intraoral malignancies. Demographically OSCC is most frequent in fifth and sixth decade of life [2].

The pathogenesis of oral cancers is multifactorial. In India and Asia, the chewing of betel quid and pan is a major regional predisposing influence since the ingredients of pan can give rise to carcinogens [1]. Smoking, a dose dependent risk is the important cause of cancer. Alcohol consumption interacts synergistically with smoking, resulting in additive risk. Smokeless tobacco in form of chewing or dipping is also considered as risk factor in certain studies [2]. HPV, particularly type 16 is recognized as etiological factor in only 3% of OSCCs. Exposure to sunlight is an established risk factor for lip cancer. Poor oral health is also associated with oral cancer. Diet rich in fruits and vegetables seems to have a protective effect against oral cancer.

Most common sites for intraoral cancer in many populations are the tongue, floor of the mouth and gingiva. In Asian population OSCCs most commonly affects buccal mucosa [2].

Small cancers may be asymptomatic. The various signs and symptoms of advanced tumours are discomfort, pain, reduced mobility of the tongue and irritation from wearing dentures. The clinical presentation is that of variably white, erythematous, mixed, nodular and ulcerated changes with raised margins. Non healing ulcer is suggestive of malignancy.

The products of most tumour suppressor genes apply brakes to cell proliferation and abnormalities in these genes lead to failure of growth inhibition another fundamental hallmark of carcinogenesis. P53 is a tumour suppressor gene located on short arm of chromosome 17 at position 17p 13.1. The p53 protein consists of 393 amino acids and comprises four regions with different functions [4]. The most common p53 alteration is a point mutation confined primarily to exons 5 to 8 [5]. P53, also called as guardian of the genome, regulates cell cycle progression, DNA repair, cellular senescence and apoptosis. It is most frequently mutated gene in human cancers [1]. It plays a central role in controlling the progression of the cell cycle from the G1- phase to the S-phase [6]. Ki-67 is a cell cycle associated human nuclear protein present in peri-chromosomal region, the expression of which strictly associated with cell proliferation and which is widely used in pathology as a proliferation marker to measure the growth fraction of cells in human tumours [7]. The estimated half-life of ki-67 antigen is 60-90 minutes. The ki-67 antigen starts to be expressed in the S phase, progressively increasing through S and G2 phases and reaching a plateau at mitosis. After cell division, the cells return to G1 with a stock of ki-67 antigen, whose level decreases rapidly during this phase [8].

## 2. Aims and objectives

- To compare demographic factors like age and sex and predisposing factors in oral squamous cell carcinomas.
- To evaluate grade of differentiation in oral squamous cell carcinoma.
- To evaluate expression of p53, Ki67 in oral squamous cell carcinomas.
- To compare the expression of p53, Ki67 in various grades of differentiation of oral squamous cell carcinomas.

## 3. Materials and methods

This was a retrospective study in which, cases of oral squamous cell carcinoma excision biopsy specimens were identified from previous records of pathology department at Government Medical College, Srikakulam. All patients had undergone part of specimen biopsy at our institute from over a period of Jan 2019 to October 2022. Informed written consent was taken from all the patients. Haematoxylin and eosin-stained slides of all cases and paraffin blocks of cases were recruited and new sections were cut when felt necessary. Slides of all cases were evaluated by two senior histopathologists independently and pathologic characteristics like tumour type and tumour grade were interpreted. Clinical records of patients were available and are thus reviewed from institutional records to evaluate patients age, sex, smoking, alcohol and gutka/pan use history, clinical features and site of biopsy. Moreover, representative tissue blocks of 35 cases were available for p53 and Ki-67 immunohistochemistry.

## 4. Interpretation results of P53 and Ki-67

The intensity of immunohistochemical staining was graded based on subjective evaluation of colour exhibited (brown colour) by antigen, antibody and chromogen complex as: negative (-, no colour), mild (+, light brown colour), moderate (++, dark brown colour) or intense (+++, very dark brown colour). Only nuclear staining of epithelial cells was observed, and the nuclei with clear brown colour, regardless of staining intensity, were regarded as positive [9]. The pattern of expression was also analysed semi quantitatively by counting the number of positive cells per 100 basal or parabasal cells and was recorded as percentage. The percentage of positive cells was scored as: 0 = 0-5%; 2 = 6-25%; 4 = 26-60%; 6 = 61-99%. The area with maximum number of positive cells was considered in each section. Normal oral mucosa is used as control.

The parameters used to analyse the expression of both p53 protein and ki-67 antigen are:

- Intensity of staining in each slide;
- The percentage of positive cells or labelling index (LI).

## 5. Results

The youngest age of presentation was 29 years and oldest age of presentation was 80 years both of which were reported as well differentiated squamous cell carcinoma, see Table 1.

Male to female ratio was 2:1.

The most common risk factor for development of cancer was seen to be smoking (57%) followed by betel quid chewing (43%).

The most common clinical presentation was identified to be non healing ulcer (80%).

Sensitivity of Ki67 and P53 is 100% and 63% respectively

**Table 1.** Age and sex correlation in oral squamous cell carcinoma

| Age | Total | Male | Female |
|---|---|---|---|
| 25-35yrs | 2(6%) | 0 | 2(100%) |
| 36-45yrs | 7(20%) | 7(100%) | 0 |
| 46-55yrs | 11(31%) | 6(55%) | 5(45%) |
| 56-65yrs | 5(14%) | 2(40%) | 3(60%) |
| 66-75yrs | 8(23%) | 6(75%) | 2(25%) |
| 76-85yrs | 2(6%) | 2(100%) | 0 |

The highest number of cases are seen in age range of 46 to 55 years. The study is showing male preponderance, see Table 2.

**Table 2.** Various sites of oral squamous cell carcinoma

| Site | Number of cases |
|---|---|
| Tongue | 16(46%) |
| Buccal mucosa | 13(37%) |
| Hard palate | 5(14%) |
| Floor of mouth | 1(3%) |

The most common site for oral squamous cell carcinoma in the study is tongue, see Table 3.

**Table 3.** Grades of differentiation of oral squamous cell carcinoma correlation with p53 expression and ki67 proliferative index:

| Grade of differentiation | Total number of cases | P53 | | Ki67 | | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | 0 | 2 | 4 | 6 |
| Well differentiated | 20(57%) | 11(55%) | 9(45%) | 0 | 2(10%) | 14(70%) | 4(20%) |
| Moderately Differentiated | 14(40%) | 10(71.4%) | 4(28.6%) | 0 | 2(14%) | 3(21%) | 9(65%) |
| Poorly differentiated | 01(3%) | 01(100%) | 0 | 0 | 0 | 0 | 1(100%) |

Most number of cases are diagnosed histochemically as well differentiated squamous cell carcinoma. P53 positivity is seen in 63% of cases in this study.Ki67 is found to be positive in 100% cases.

The photomicrograph of P53 immunohistochemical stain negativity is shown in Figure 1, while the photomicrograph of P53 immunohistochemical stain showing nuclear positivity is dispatched in Figure 2. Also, the photomicrograph of P53 immunohistochemical stain showing nuclear positivity is presented Figure 3 and the photomicrograph of Ki67 immunohistochemical stain illustrating grade 2 positivity with 16% positivity is given in Figure 4. The photomicrograph of Ki67 immunohistochemical stain illustrating grade 4 positivity with 55% positivity is shown in Figure 5 and the photomicrograph of Ki67 immunohistochemical stain illustrating grade 6 positivity with 80% positivity is given in Figure 6.

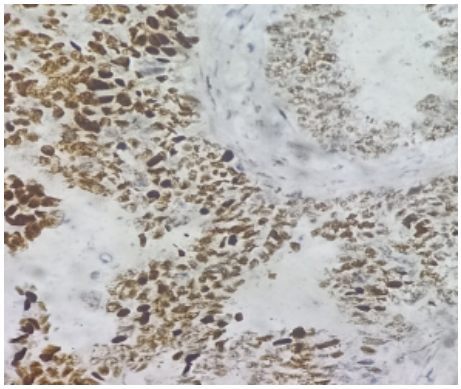

**Figure 1.** Photomicrograph of P53 immunohistochemical stain negativity (40x view)

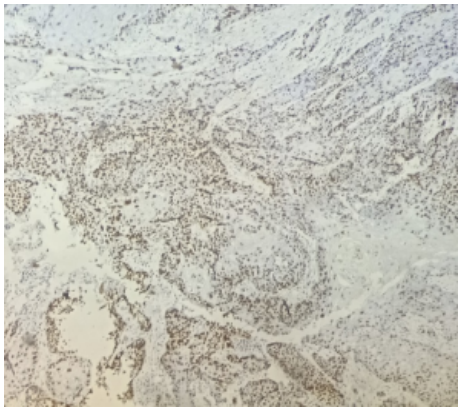

**Figure 2.** Photomicrograph of P53 immunohistochemical stain showing nuclear positivity (in 10x view)

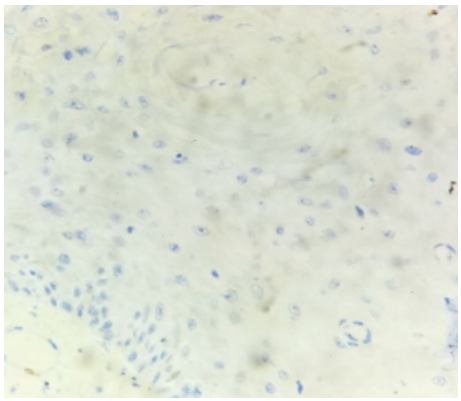

**Figure 3.** Photomicrograph of P53 immunohistochemical stain showing nuclear positivity (40X view)

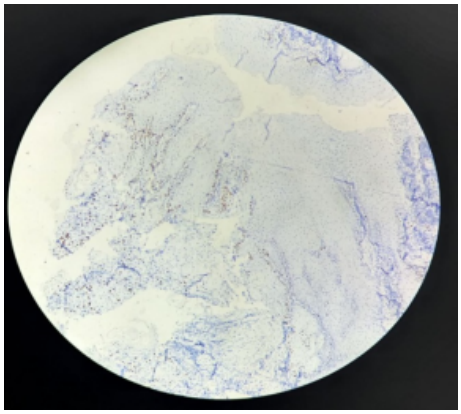

**Figure 4.** Photomicrograph of Ki67 immunohistochemical stain illustrating grade 2 positivity with 16% positivity (10x view)

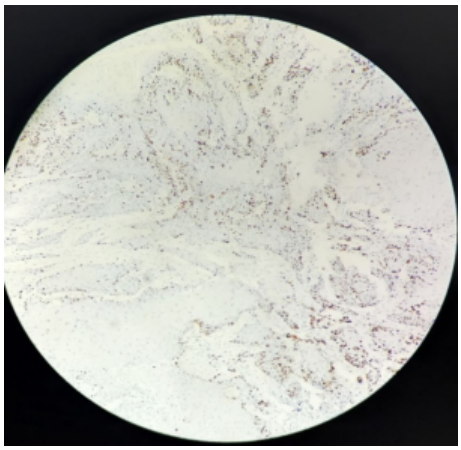

**Figure 5.** Photomicrograph of Ki67 immunohistochemical stain illustrating grade 4 positivity with 55% positivity (10x view)

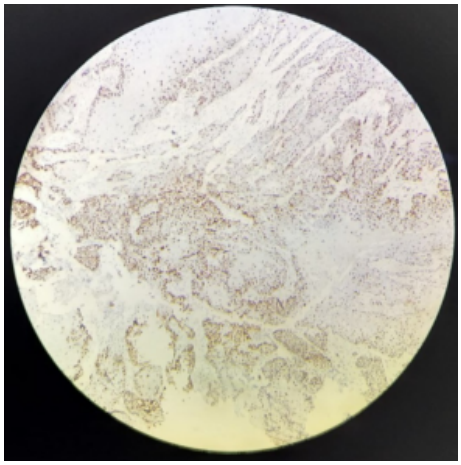

**Figure 6.** Photomicrograph of Ki67 immunohistochemical stain illustrating grade 6 positivity with 80% positivity (10x view)

## 6. Discussion

The maximum age of presentation of OSCCs is between 46-55 years (31%). This is quite similar to studies conducted by Patel *et al.,* [10] and Dragomir *et al.,* [11] and in study conducted by Maheshwari *et al.,* [9] the

highest age of presentation was between 61-70 years, this variation could be due to small sample size and variation in geographic distribution.

The male dominance can be due to increased practice of smoking and betel quid chewing, predisposing factors for OSCCs in males. The male to female ratio in present study is 2:1. This is in conderance with study done by similar to Kaur *et al.,* [12]. In studies done by Maheshwari *et al.,* [9], Dragomir *et al.,* [11] also show male preponderance, see Table 4.

**Table 4.** Male to female ratio in co-ordnance with other studies

| Studies | Male to female ratio |
|---|---|
| Maheshwari *et al.,* [9] | 4:1 |
| Kaur *et al.,* [12] | 2:1 |
| Dragomir *et al.,* [11] | 5:1 |
| Present study | 2:1 |

Most common site for development of OSCCs is tongue. The variations in sites have been proposed to be related to racial differences and varying environmental risk factors In Oral Cancers [14]. This is similar to the studies done by Patel *et al.,* [10], Maheshwari *et al.,* [9], Raju *et al.,* [13], see Table 5.

**Table 5.** Site of tumour in relation with other studies

| Studies | Location |
|---|---|
| Patel *et al.,* [10] | Tongue (48.71%) |
| Maheshwari *et al.,* [9] | Tongue (41.54%) |
| Raju *et al.,* [13] | Tongue (37%) |
| Present study | Tongue (46%) |

Mutations in the p53 gene are the most common genetic changes observed in OSCCs. They lead to uncontrolled cell proliferation, resulting in further genetic abnormalities and finally malignancy[16]. In this study 57% cases are well differentiated, 40% are moderately differentiated, 3% are poorly differentiated. The rate of differentiation is one of the factors that determines the prognosis of patient. This finding is consistent with study done by Khan *et al.,* [15] and Dragomir *et al.,* [11] where well differentiated OSCCs are 46.7% and 52.9% respectively. In study done by Dragomir *et al.,* [11] and Kaur *et al.,* [12], moderately differentiated carcinoma accounts for 35.3% and 35.8% respectively. In study done by Khan *et al.,* [15] and Maheshwari *et al.,* [9], poorly differentiated carcinoma accounts for 8.3% and 7.69%, see Table 6.

**Table 6.** Grade of differentiation in other studies

| Studies | Grade of differentiation | | |
|---|---|---|---|
| | Well differentiated | Moderately differentiated | Poorly differentiated |
| Khan *et al.,* [15] | 46.7% | 45% | 8.3% |
| Dragomir *et al.,* [11] | 52.9% | 35.3% | 11.8% |
| Maheshwari *et al.,* [9] | 30.76% | 23.07% | 7.69% |
| Kaur *et al.,* [12] | 43.4% | 35.8% | 20.8% |
| Present study | 57% | 40% | 3% |

To analyse the proliferative status of a cell or tissue Ki-67 marker is reliable and widely used. It recognizes a proliferation-related nuclear antigen present at all phases of cell cycle except G0. The positivity of P53 in this study is 63%. This is in accordance with the studies done by Ara *et al.,* [17], Hashmi *et al.,* [18], Ghanghoria *et al.,* [19], see Table 7.

**Table 7.** P53 positivity with other studies

| Studies | P53 positivity |
|---|---|
| Ara *et al.,* [17] | 67% |
| Hashmi *et al.,* [18] | 66.1% |
| Ghanghoria *et al.,* [19] | 63% |
| Present study | 63% |

Co-expression and correlation between p53 and Ki-67 have been demonstrated, suggesting that alterations in the p53 protein might lead to increased cell proliferation. Furthermore, over expressions of p53 and Ki-67 have been suggested to be reliable indicators for Oral Cancer development [14]. The positivity of Ki67 is 100%. This is in concurrence with the study done by Maheshwari *et al.,* [9], Dragomir *et al.,* [11], see Table 8.

**Table 8.** Ki67 positivity with other studies

| Studies | Ki67 positivity |
|---|---|
| Raju B *et al.,* [13] | 91% |
| Maheswari V *et al.,* [9] | 100% |
| L. P. Dragomir *et al.,* [11] | 100% |
| Present study | 100% |

## 7. Conclusion

The present study demonstrated the highest number of cases to be well differentiated squamous cell carcinoma. P53 positivity was demonstrated in majority of cases indicating that it is most common genetic mutations in oral cancers. The present study showed an inverse correlation between the degree of tumour differentiation and the rate of cell proliferation obtained by the expression of Ki-67.

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**Conflicts of Interest:** "Authors declare that they do not have conflict of interests."

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