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**ORIGINAL ARTICLE**

# Study of Immunohistochemical Expression of p53 and Ki-67 in Oral Squamous Cell Carcinoma

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## Abstract

**Objective: "**Study of Immunohistochemical Expression of p53 and Ki-67 in Oral Squamous Cell Carcinoma" **Methods**: The descriptive analysis, which was based in a hospital, comprised 60 cases of oral squamous cell carcinoma (OSCC). Cases presented at Muzaffarnagar Medical College, Pathology department. Immunohistochemically, p53 and Ki-67 expression were assessed and correlated with clinicopathological characteristics. **Results**: The Majority of cases having OSCC were in fifth and sixth decades. 'The tongue is the most common site for OSCC.' Tobacco use was present in 68.3% of patients. Most OSCC instances were moderately differentiated. p53 and Ki-67 expression were substantially higher in poorly differentiated patients. No significant correlation was seen between age, gender, or histological grade and 56.7% positive p53 expression. A strong correlation existed between age, histological grade, and Ki-67 expression. **Conclusion: '**The study highlights the importance of understanding oral squamous cell carcinoma patients' habits and characteristics for improvement of prognosis as well as the treatment options. These markers are recommended for assessing OSCC aggressiveness and progression, but further research is needed to understand their roles in treatment outcomes by incorporating a broader panel of markers.'

**Keywords:** Oral squamous cell carcinoma, Immunoexpression, p53, Ki-67

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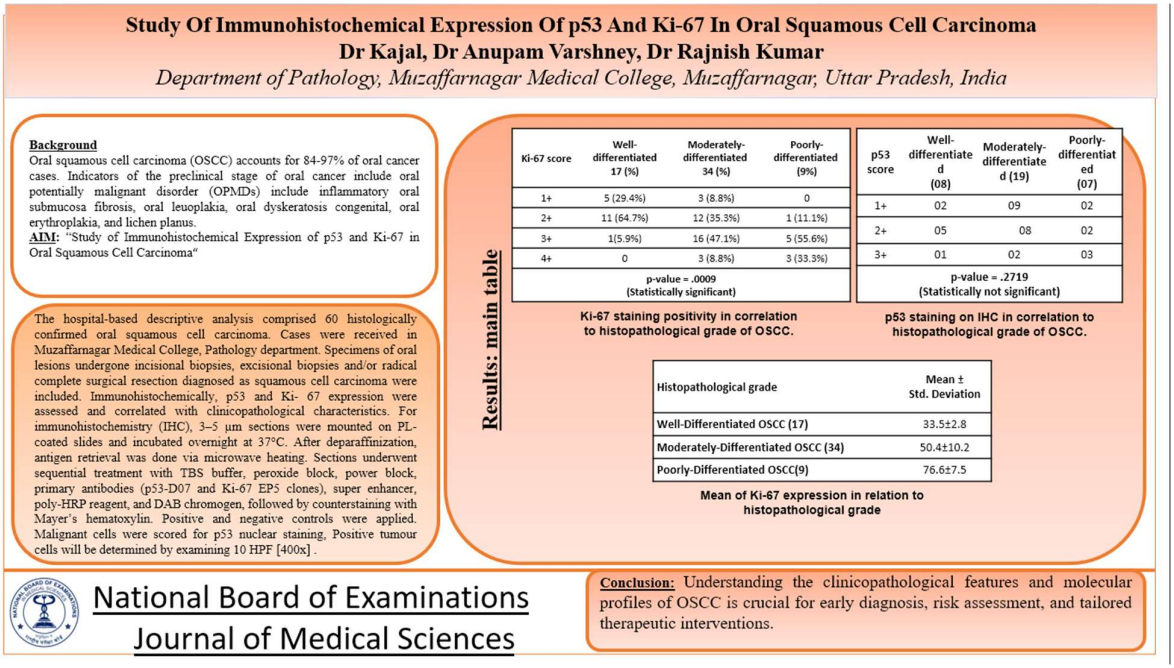
## Graphical Abstract




## Introduction

Oral carcinoma is considered one of the most common malignancies worldwide, especially in underdeveloped countries. It is a prominent cause of death in India. In India, HPV causes 40% of oral malignancies, compared to 2–4% in Western countries. OSCC prognosis is poor due to delayed diagnosis, despite advances in diagnostic methods. Early identification can boost 5-year survival rates by 80% and improve quality of life [1].

GLOBOCAN 2022 data from the "International Agency for Research on Cancer (IARC)" reported 20million new cancer cases and 9.7million deaths worldwide. Lip and oral cancers were 16th in incidence and 15th in mortality, with 389,485 (2%) new cases and 88,230 (1.9%) deaths [2]. In India, oral cancer led to 75,290 deaths in 2020, accounting for 5.4 deaths per 100,000 population [3]. Central and Southeast Asia, including India, have high age-standardized incidence rates of 14.8/100,000 men and 4.6/100,000 women [3].

Tobacco and alcohol use are major contributors to the multifactorial etiology of oral cancer, causing genetic and molecular alterations that lead to premalignant lesions [4]. Biomarkers at the molecular level can enhance early diagnosis and prognostic evaluation [5]. Leukoplakia and oral submucous fibrosis (OSMF) are common premalignant lesions that frequently appear before OSCC [1]. Leukoplakia, characterized by white oral mucosal patches, progresses to squamous cell carcinoma (SCC) in 5–43% of cases, depending on dysplasia severity [6].

Tumors are graded using Broder's classification based on differentiation and keratinization: Grade I >75% "differentiated), Grade II (>50 to 75%) and Grade III (25 to 50%") [7]. The TP53 gene encodes p53, a tumor suppressor that prevents genetic damage through DNA repair or apoptosis [8]. Mutations in p53, especially in exons 5–8, compromise this function, promoting tumor growth [9]. A

nuclear protein marker called Ki-67 is frequently used to measure tumour growth since it corresponds with cell proliferation [10,11]. This study aims to assess p53 and Ki-67 expression in OSCC using immunohistochemistry (IHC) in India. Emphasis on early detection and prevention remains critical [12].

**Material and Methods**

The present study, titled "Study of Immunohistochemical Expression of p53 and Ki-67 in Oral Squamous Cell Carcinoma," had been performed in the Department of Pathology, "Muzaffarnagar Medical College, Muzaffarnagar (U.P.)". This was a hospital record-based descriptive study for 18 months, including 60 cases of histologically diagnosed OSCC. Both retrospective and prospective samples were examined.

*Inclusion criteria:* Squamous cell carcinoma was identified in oral lesion specimens that underwent radical total surgical resection, excisional biopsies, or incisional biopsies.

*Exclusion criteria:* Tissues lacking viable or representative material. Tissue biopsies of patients treated with radiation therapy and/or chemotherapy after being diagnosed with oral squamous cell carcinoma. Following their fixation in 10% buffered formalin, the samples were embedded in paraffin, processed by employing a semi-automated tissue processor (Thermo Scientific MICROM STP 120), and sectioned at 3–4µm by employing a rotary microtome (Thermo Scientific MICROM HM 325). The normal protocols for H&E (haematoxylin and eosin) staining were followed [13].

For IHC 3–5 µm sections had been mounted on PL-coated slides and incubated overnight at 37°C. After deparaffinization, antigen retrieval was done via microwave heating. TBS buffer, peroxide block, power block, primary antibodies (p53-D07 and Ki-67 EP5 clones), super enhancer, poly-HRP reagent, and DAB chromogen were applied to the sections one after the other. Mayer's haematoxylin was then used as a counterstain. Both negative and positive controls were used. Malignant cells were scored for p53 nuclear staining. Positive tumour cells will be determined by examining 10 HPF [400x] [14] (Table 1).

Table 1. p53 scoring

| Score | Percentage |
|---|---|
| **0** | Less than 10% of malignant cells positive |
| **1+** | 10 to 30% of malignant cells are positive |
| **2+** | 31 to 50% of malignant cells are positive |
| **3+** | >50% of malignant cells are positive |

Malignant cells had been scored for Ki-67 nuclear staining [Labelling index], positive cells will be determined by examining 10 HPF [400x] [15] (Table 2).

Table 2. Ki-67 scoring

| Score | Percentage |
|---|---|
| 1+ | LI=1% to25%of malignant cells positive |
| 2+ | LI=26% to 50%of malignant cells positive |
| 3+ | LI=51% to 75% of malignant cells positive |
| 4+ | LI=>75% of malignant cells are positive |

MedCalc software version 22.020 [16] was employed for statistical analysis and MS Excel was used to generate the data. "Ethical clearance had been obtained from the Institutional Ethics Committee" of Muzaffarnagar Medical College and informed consent had been taken from all patients. Observations were presented in tables and figures and analyzed using relevant statistical tools.

## Results

For eighteen months, this descriptive retro-prospective study had been performed in the pathology department of "Muzaffarnagar Medical College and Hospital". Following ethical approval and established inclusion/exclusion criteria, sixty cases of OSCC with histological confirmation were included. Haematoxylin and Eosin (H&E) staining, standard processing, and tissue fixation were all performed. The expression of p53 and Ki-67 markers in tumour cells was evaluated by immunohistochemistry (IHC).

### Demographic Characteristics

The average age of the patients was 50.68 ± 11.59yrs., with a range of 30 to 80 years. The fifth decade noted to have highest incidence of 46.7 % followed by sixth decade (18.3%). With a male-to-female ratio of 5:1, males accounted for 83.3% of cases and were the most affected (Figure 1).

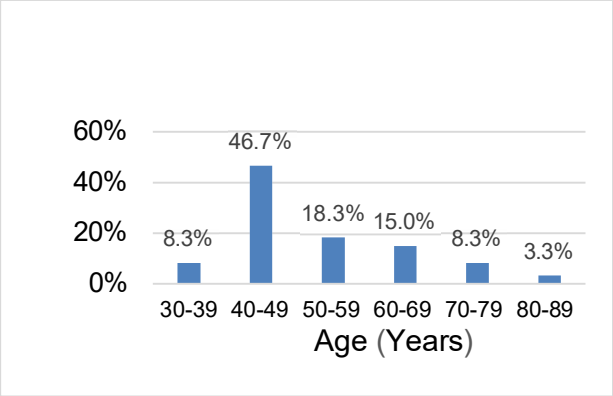



Figure 1. Bar chart showing age-wise distribution of OSCC (%)

**Site Distribution**

The tongue, especially the left lateral border, was the most frequently impacted region (36.6%). The gingiva (10%) and buccal mucosa (35.0%) came next. Other less common sites involved the hard palate, lip, floor of the mouth, angle of mouth, and retromolar trigone (Figure 2).

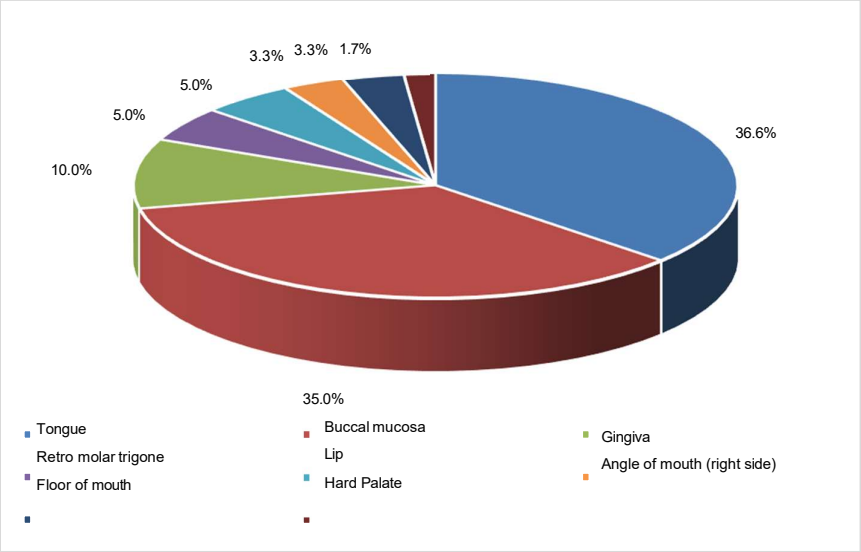



Figure 2. Pie chart showing distribution of OSCC according to sites

**Tobacco Use**

Tobacco users were categorized into three groups: Only tobacco chewers, only smokers, and both chewers as well as smokers. A significant number of OSCC patients (68.3%) had a history of tobacco use—30% used both chewers as well as smokers. 25% were smokers only, and 13.3% were tobacco chewers only. Non-users comprised 31.7%.

**Specimen and Gross Morphology**

Of the specimens, 56.7% were incisional biopsies, and 43.3% were excisional. On gross examination, the ulcero-proliferative type was most common (45%), followed by proliferative (33.3%) and ulcerative (21.7%) types.

**Histopathological Grading**

Moderately differentiated OSCC was the most common histological grade (56.7%), followed by 28.3% (well-differentiated), and 15% of poorly differentiated type of OSCC. A correlation was observed between higher-grade tumors and use of both tobacco chewers and smokers (Figures 3 to 6).

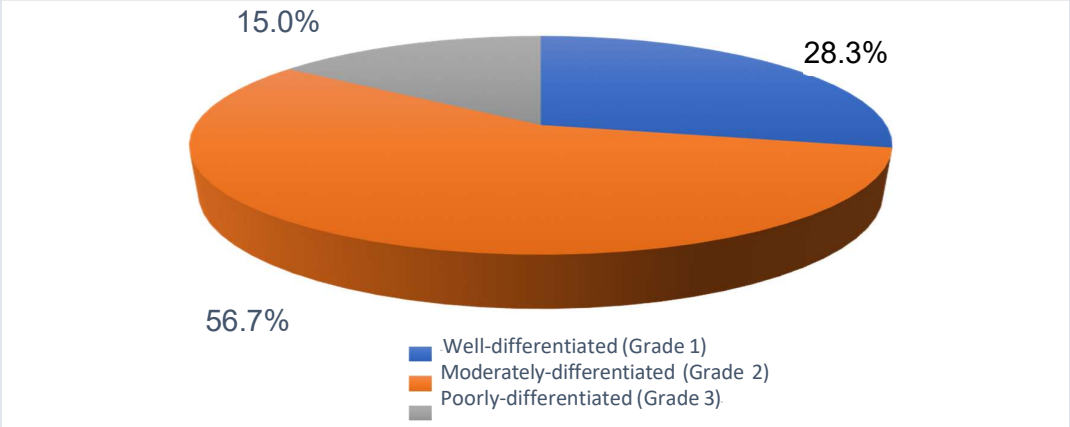



Figure 3. Pie chart showing distribution of study subjects according to histological grades of OSCC

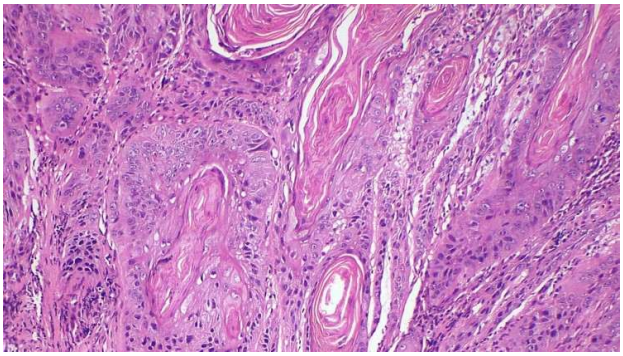


Figure 4. Photomicrograph of Well Differentiated "Oral Squamous Cell Carcinoma" (H&E 100x)

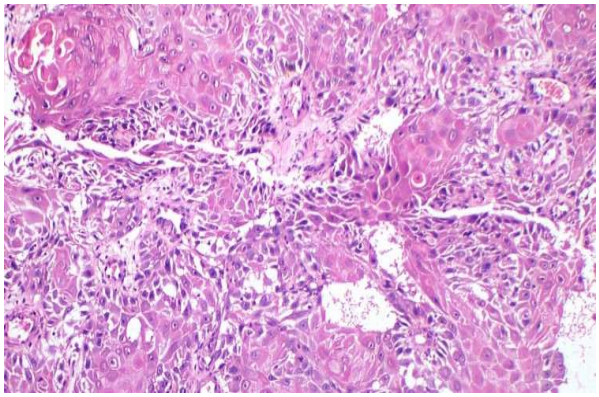


Figure 5. Photomicrograph of Moderately Differentiated "Oral Squamous Cell Carcinoma" (H&E 100x)

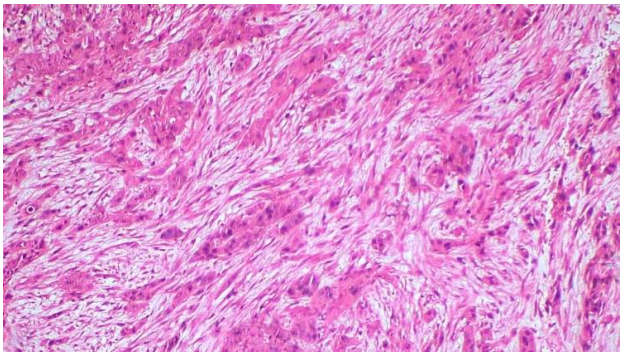


Figure 6. Photomicrograph of Poorly Differentiated OSCC ("Oral Squamous Cell Carcinoma") (H&E 100X)

**p53 Immunohistochemistry**

p53 expression on IHC was positive in 56.7% of cases. The most frequent immune-expression was 2+ 15/34(44.1%), followed by 1+ 13/34(38.2%) and 3+ 6/34(17.6%). Positive p53 expression was slightly more prevalent in patients over 50 years (66.7%) compared to those 50 years or younger (50%). Males showed a higher rate of positivity (58%) than females (50%), although statistical analysis did not show significance ($p > 0.05$). Poorly differentiated OSCC had the greatest p53 positivity among histological grades (77.8%), followed by moderately differentiated (55.9%) and well-differentiated (47%). However, these differences were also statistically nonsignificant (Figures 7 to 10).

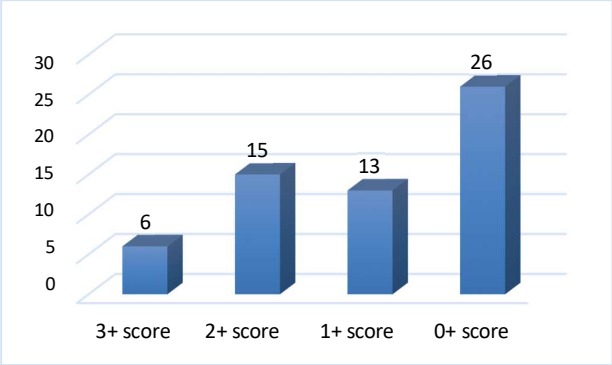



Figure 7. 3D column chart showing total number of cases in p53 score on IHC.

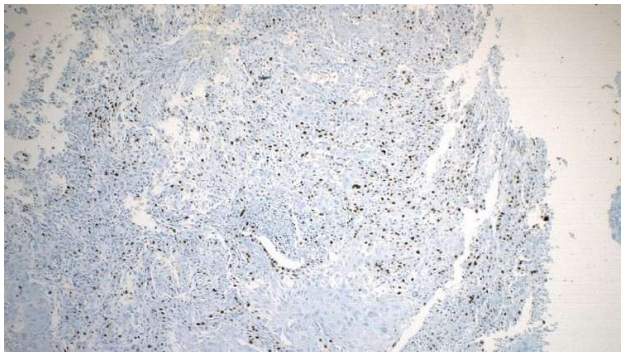


Figure 8. Photomicrograph showing immune-positivity for p53 score 1 (IHC 400x)

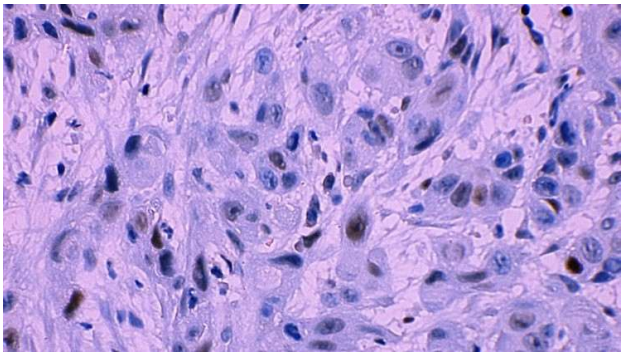


Figure 9. Photomicrograph displaying immunopositivity for p53 score 2 (IHC 40x)

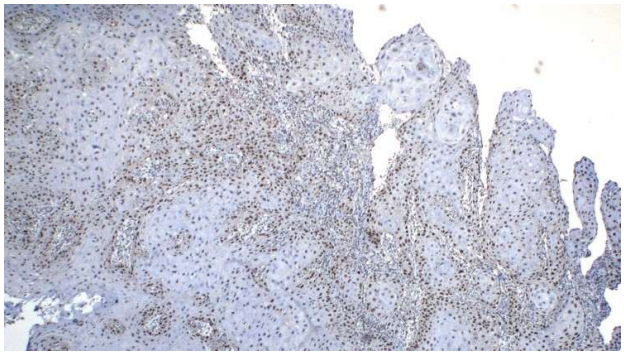


Figure 10. Photomicrograph showing immunopositivity for p53 score 3 (IHC 40x)

**Ki-67 Immunohistochemistry**

Ki-67 expression on IHC was evaluated. Most patients had moderate 2+ 24/60(40%) to high 3+, 22/60(36.7%) expression. Younger patients (≤50 years) had slightly higher Ki-67 positivity. Among histological grades, poorly differentiated OSCC showed the highest Ki-67 expression (55.5% had 3+ or 4+), indicating a correlation with tumor aggressiveness. Well-differentiated OSCCs mainly showed lower scores (1+ and 2+) (Figures 11 to 14).

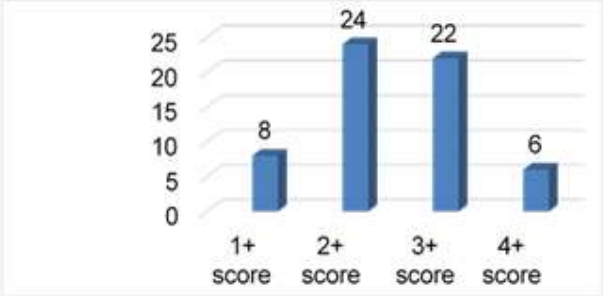



Figure 11. 3D column chart showing total number of cases in Ki-67 score

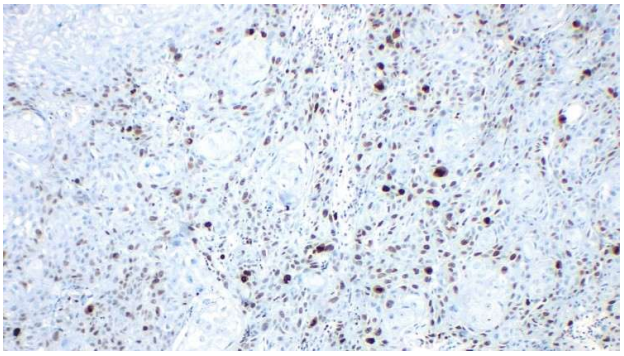


Figure 12. Photomicrograph showing immune positivity for Ki-67 score 2 (IHC 40x)

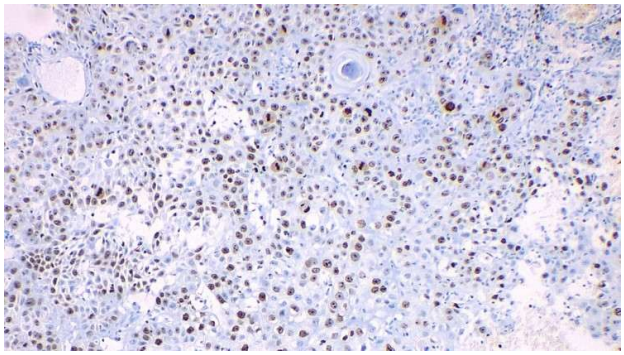


Figure 13. Photomicrograph showing immune positivity for Ki-67 score 3 (IHC 100x)

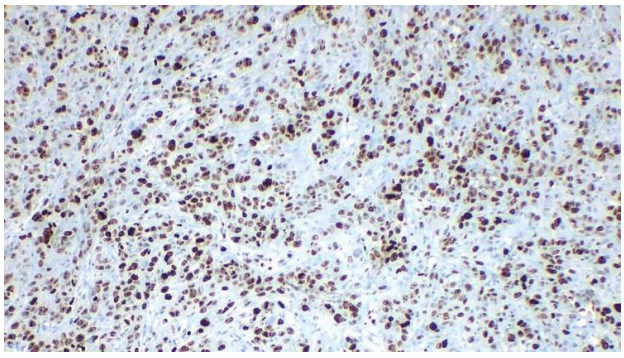


Figure 14. Photomicrograph showing immune positivity for Ki 67 score 4 (IHC 100x)

**Discussion**

In India, OSCC continues to be a major health burden, and its aetiology is significantly influenced by lifestyle factors including tobacco use. This study, conducted at "Muzaffarnagar Medical College in Uttar Pradesh", has improved our understanding of the clinic-pathological and immune histochemical characteristics of OSCC. With a male-to-female ratio of 5:1, the disease primarily affects males in their 5th and 6th decades of life, according to a review of 60 histologically verified OSCC cases. The results were in line with those of Sahaf et al. [18] whose mean patient age was 51.8 years and Yasin et al. [17] whose mean diagnostic age was 48 years. Our study revealed a male preponderance in OSCC, which was consistent with results by Singh MP et al. [19] and Chandrakanta et al. [20].

The buccal mucosa, tongue, as well as gingiva were the most often affected anatomical areas, which is consistent with the areas' typical of tobacco chewing. The results aligned with research performed by Singh et al. [19], which observed that the buccal mucosa was the highly affected area in OSCC and that a history of tobacco use was also prevalent. Our findings were similar to those of Chandrakanta et al. [20]. It found that the tongue was the most frequently observed site (50%), followed by the buccal mucosa (18.42%).

The majority of the cases had moderately differentiated tumors, followed by well-differentiated and then poorly-differentiated tumors. The investigation closely resembles research by Yasin et al. [17], which found that moderately differentiated OSCC had the highest number of instances, well-differentiated OSCC had the second-highest number, and poorly differentiated OSCC had the least. The results contrasted with research by Sahaf et al. [18], who found that a large proportion of OSCC was well-differentiated, followed by OSCC that was moderately differentiated, and finally OSCC that was poorly differentiated.

The immune-histochemical analysis for p53 provided important insights into tumor biology. Our study found that p53 expression increased as histopathological grade increased. This result is in close agreement with the discoveries of Mohanapure et al. [15] regarding p53 expression, which showed that p53 expression was positive in 44% of well-differentiated OSCC, 53.8% of moderately-differentiated carcinoma, and 78.9% of poorly-differentiated carcinoma. We can infer that the histological OSCC grade significantly increases the p53 expression. According to a study by Singla et al. [14], p53 positivity was 100% in cases of moderately and poorly differentiated oral squamous cell carcinoma since no case was p53 negative in these situations (Table 3).

Table 3. Comparison between p53 positivity with scoring and histological grading

| | | **Present study** | Mohanapure et al 2022 [15] | Singla et al. 2016 [14] |
|---|---|---|---|---|
| **Well differentiated** | **1** | **25%** | 36.4% | 66.7% |
| | **2** | **62.5%** | 63.6% | 25.0% |
| | **3** | **12.5%** | 0% | 0% |
| **Moderately differentiated** | **1** | **47.3%** | 50% | 42.1% |
| | **2** | **42.1%** | 42.9% | 31.6% |
| | **3** | **10.5%** | 7.1% | 26.3% |
| **Poorly differentiated** | **1** | **28.5%** | 46.6% | 0% |
| | **2** | **28.5%** | 26.7% | 11.1% |
| | **3** | **42.8%** | 26.7% | 88.8% |

The mean Ki-67 LI in the current study was significantly higher for poorly differentiated individuals (76.6%±7.4%) than for moderately differentiated individuals (50.4%±10.2%), which was significantly higher than for well-differentiated individuals (33.5%±2.8%). The results aligned with the research conducted by Chanadrakanta et al. [20], which found that the KI-67 LI was 28.5% in well-differentiated OSCC, 42.8% in moderately-differentiated carcinoma, and 68.5% in poorly-differentiated carcinoma.

Both p53 and Ki-67 markers showed increased expression in poorly differentiated tumors, Ki-67 exhibited a statistically significant correlation with tumor grade, reflecting its role as a proliferation marker. On the other hand, p53 expression, though elevated in more aggressive tumors, did not show a significant association with age, gender, or histological grade, suggesting that its role in OSCC may be more complex and influenced by additional genetic or environmental factors.

These results demonstrate the potential value of Ki-67 as a trustworthy biomarker for determining the aggressiveness of tumours and forecasting OSCC proliferative behaviour. The variable expression of p53, although not statistically significant in this cohort, indicates a possible involvement in tumor progression that warrants further investigation. Together, these markers may aid in better prognostication and could inform personalized treatment strategies in the future.

## Conclusion

In conclusion, understanding the clinic pathological features and molecular profiles of OSCC is crucial for early diagnosis, risk assessment, and tailored therapeutic interventions. This work contributes to the increasing amount of data demonstrating the utility of

immunohistochemistry markers in the assessment of OSCC. Further large-scale, multi-centric studies incorporating a broader panel of biomarkers are recommended to deepen our understanding of OSCC pathogenesis and improve patient outcomes.

## Statements and Declarations

### Conflicts of interest

The authors declare that they do not have conflict of interest.

### Funding

No funding was received for conducting this study.

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