**Research Article**

# Mitotic Index and its Role in Squamous Cell Carcinoma of Oral Cavity

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## I N F O

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## A B S T R A C T

*Background:* Oral cavity carcinomas include lips, buccal mucosa, gingiva, anterior two-third of tongue, floor of the mouth and hard palate. In cancer, atypical mitosis and its increase indicates genetic damage in tumours and loss of controlled proliferation. Therefore, identification and quantification of mitosis forms an important part of the histological grading systems.

*Materials and Methods:* The sample of the study included 37 H&E stained slides diagnosed for various grades of Oral Squamous Cell Carcinoma from the archives of the Department of Pathology, Sharda University, School of Medical Science and Research, Uttar Pradesh, India. The sections were analysed for mitosis under a light microscope, under 40x.

*Results:* Out of the 37 cases, four cases were Carcinoma in Situ, 16 cases were well-differentiated squamous cell carcinoma, 12 cases were moderately differentiated squamous cell carcinoma and five cases were poorly differentiated squamous cell carcinoma. It was found that the mean mitotic count in males was 4.17 and females was 2.88. This was statistically insignificant. The mean mitotic count of buccal mucosa and tongue was 3.42, 3.49 respectively, which was insignificant. The mean mitotic count in carcinoma in situ, well differentiated, moderately differentiated and poorly differentiated was 5.00±6.880, 3.31±3.281, 4.50±4.661, 3.40±3.782 respectively, which came out to be statistically insignificant.

Conclusion: Methods of detecting tumour type in oral cavity and prognostication should be supplemented with Ki67 or other immunohistochemistry markers as has been done in luminal classification of breast carcinoma.

**Keywords:** Squamous Cell Carcinoma, Mitotic count, Oral Cancer

## Introduction

Oral cancer (cancer of lips, mouth and tongue) is defined as a malignant neoplasia on the lip or oral cavity.[1] The carcinomas included in the oral cavity are of the lips, buccal mucosa, gingiva, anterior two-third of tongue, floor of the mouth and hard palate.[2] This form of cancer is the sixth most common cancer worldwide, and the third most

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common form of malignancy in developing countries.[3] It has a global incidence of 350,000 new cases per year and 177000 deaths every year.[4] In India specifically, oral cancer accounts for 10% of all cancer cases, 95% being squamous cell carcinoma.[5] The mean age is said to be 50-60 years and increases in incidence with age. However, several epidemiology studies have shown a rising trend in young age groups.[6] It is common in males, a research study showed 68.90% occurred in males, and 1911 cases (31.07%) were diagnosed in females. The male-to-female ratio was 2.22:1.[7] The main causative agents are first and second hand smoking and alcohol, which constitute 90% cases. Other risk factors are mutagens such as UV radiations and human papillomavirus associated with the oral carcinoma.[1] In carcinomas excess proliferation of cells is a major hallmark of cancer and pre-cancer. Moreover, atypical mitosis and its increase indicates genetic damage in tumours and loss of controlled proliferation ultimately leading to cancer formation. Thus, identification and quantification of mitosis forms an important part of the histological grading systems used for the prognostication of precancerous and cancerous lesions and so, traditional approaches in histopathological grading are focused on mitotic activity and depth of invasion.
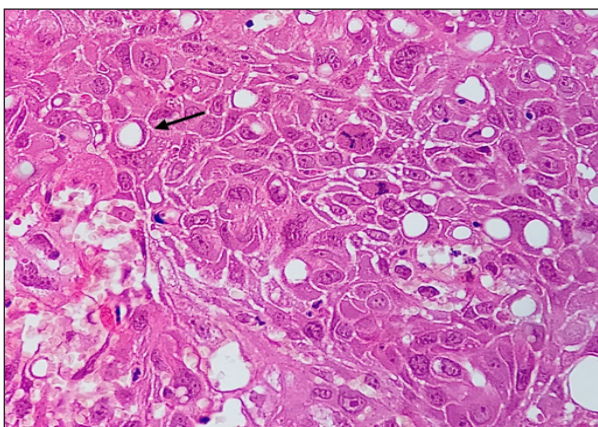


**Figure 1.Photograph of oral Squamous cell Carcinoma Showing Atypical Mitosis (H&E stained, 40X)**

## Materials and Methods

All the cases, which were biopsied from the oral cavity during the period of 1st January 2019 to July 2022, were registered for the study. Their clinical details were collected, the histopathology slides were reviewed, and the final diagnosis was confirmed.

The study sample included retrieval of 37 H&E stained slides diagnosed for various grades of Oral Squamous Cell Carcinoma from the archives of the Department of Pathology, Sharda University, School of Medical Science and Research, Uttar Pradesh, India. Wherever needed, the paraffin blocks were taken out and further sections

were studied. Study samples of 37 diagnosed cases of oral squamous cell carcinoma were grouped into four categories:

Group 1: Carcinoma in-situ, Group 2 - Well differentiated, Group 3: Moderately differentiated and Group 4 - Poorly differentiated.

H&E sections were analysed for mitotic figures under 40x, under a light microscope. The criteria provided by Diest V et al. were strictly used to identify mitotic figures.[8]

The entire thickness of the epithelial lining was critically analysed and the basement membrane invasion was noted. For counting the mitotic rate, five fields were chosen in a slide. The fields were in four quadrants and the central quadrant of the tissue to have a wider representation. A total of 500 cells were counted out of which the mitotic rate per 10 high power fields was calculated.

The data was analysed using the student t test and a p value ≤ 0.05 was taken as significant. This was done with the help of SPSS v-21.

## Result

All The cases coming to the department of Pathology were listed. Out of all these cases, the Oral squamous cell carcinoma that were registered for the study were taken out and reviewed. There were 37 cases of Oral squamous cell carcinoma out of which four cases were Carcinoma in Situ, 16 cases were well-differentiated squamous cell carcinoma, 12 cases were moderately differentiated squamous cell carcinoma and five cases were poorly differentiated squamous cell carcinoma.

**Table 1.Mitotic Rate and Gender**

| Gender | Mitosis count in 10 field | | | |
|--------|------|------|---------|---------|
| | **Mean** | **S.D** | **t-Value** | **P value** |
| Male | 4.17 | 4.376 | 0.7769 | 0.4424 |
| Female | 2.88 | 3.137 | | |

**Table 2.Mitotic Rate and different Sites of Carcinoma**

| Site | Mitosis count in 10 field | | | |
|------|------|------|---------|---------|
| | **Mean** | **S.D** | **t value** | **P value** |
| Buccal mucosa | 3.42 | 4.073 | 0.7082 | 0.4835 |
| Tongue | 4.39 | 4.258 | | |

An attempt was made to compare gender with the mean mitotic count (in 10 fields). It was found that the mean mitotic count in males was 4.17 and females was 2.88. The mean mitotic count was more in males than in females. The t-value was found out to be 0.7769 and the P value was 0.4424. Thus, Table 1, demonstrates that the results were statistically insignificant.

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**Table 3.Mitotic Count and Differentiation of Squamous cell Carcinoma**

| Types of Differentiation | Mitosis count in 10 field | |
|---|---|---|
| | Mean | S.D |
| Carcinoma in situ | 5.00 | 6.880 |
| Tongue | 3.31 | 3.281 |
| Squamous cell Carcinoma-Moderately Differentiated | 4.50 | 4.661 |
| Squamous Cell Carcinoma-Poorly Differentiated | 3.40 | 3.782 |

Mitotic count was further analysed according to the site. There were 18 cases in buccal mucosa and 19 cases in tongue. The mean mitotic count of buccal mucosa was 3.42 and the mitotic mean of tongue was 3.49. The t- value came out to be 0.7082 (P=0.4835). Statistically these results were not significant (Table 2).

The types of differentiation were compared with the mean mitotic count as shown in Table III. The study revealed that the highest mean mitotic count was seen in Carcinoma in Situ which was 5.00, followed by moderately differentiated squamous cell carcinoma which was 4.50 and poorly differentiated squamous cell carcinoma, which was 3.40. The lowest mitotic count was seen in well differentiated squamous cell carcinoma which was 3.31. Upon comparison of carcinoma in situ and moderately differentiated squamous cell carcinoma, the t value came out to be 0.166 (P value=0.875). The comparison of poorly differentiated squamous cell carcinoma with carcinoma in situ gave a t value of 0.447 (P value= 0.6683). The t value for the comparison of carcinoma in situ and well-differentiated squamous cell carcinoma was 0.736 (P value=0.4711). The comparison of moderately differentiated and poorly differentiated squamous cell carcinoma gave a t value of 0.465 (P value=0.6486). The t value for comparing well and moderately differentiated squamous cell carcinoma was 0.794 (P value=0.4344). Lastly, upon comparison of poorly and well differentiated squamous cell carcinoma, the t value was 0.052 (P value=0.9592). Thus, the mean difference in mitotic values in the different types of differentiation of squamous cell carcinoma was statistically not significant.

## Discussion

Carcinogenesis occurs when nuclear DNA undergoes genetic mutations resulting in dysregulation of mitosis.[9] Increased and atypical mitosis seen in oral squamous cell carcinoma indicates genetic mutation and has a significant role in carcinogenesis.[10] Similar criteria was used as an important parameter for grading of breast cancer cases under Bloom-Richardson grading system. It is a system that examines the cell and tissue to evaluate how aggressive and invasive the tumour is based on mitotic index, tubule formation and nuclear pleomorphism.[11]

The classification of smooth muscle tumour into benign, borderline and malignant is based on rate of mitosis. Therefore, evaluation of mitotic activity under a microscope seems to be an important prognostic indicator. It is known that the significance of mitotic figures is helpful in assessing prognosis of various grades of cancer, cause of chromosomal aberration and aids in histological grading by evaluating cellular proliferation.[12]

Irrespective of the site, squamous cell carcinoma evolves through a sequence of changes occurring in the normal squamous epithelium, ranging from dysplasia of varying degrees to clinically evident malignancy, which ultimately infiltrates the basement membrane and causes metastasis. It is thought that this varying degree of aggressiveness of the malignant cell depends on their proliferative rate. Thus, higher the proliferative rate, potential is its ability to infiltrate and metastasize, and cause poor prognosis. This proliferative rate can be estimated by monoclonal antibodies such as Ki67 or by titrated thymidine labelling.[13] However, the simplest and cheapest method is by counting the mitotic rate under a light microscope.

Thus, this study was undertaken to find out the exact role of mitosis in predicting the type of squamous cell carcinoma of oral cavity. Aetiologically, it is different in Indian population compared to the western population, and if so then this not only becomes a simple and a good marker for clarifying the type of oral squamous cell carcinoma but also a prognostic indicator.

The present study revealed that the mean mitotic count per 10 high power field (hpf) in carcinoma in situ, well differentiated, moderately differentiated and poorly differentiated was 5.00±6.880, 3.31±3.281, 4.50±4.661, 3.40±3.782 respectively. However, this did not show any statistically significant difference. Similar findings have also been reported by Dr. Karan et al, who found that mitotic cell counting is notoriously unreliable.[12]

It is known that the stimulus to cell proliferation starts at the 'S' phase of the cell cycle. The cell then passes through the G2 phase of the cell cycle and enters mitotic phase 'M'. This cell cycle takes around 24 hours and the mitotic phase itself occupies only 20 minutes to 1 hour of this cycle. However, the major part of the cell cycle is taken up by DNA synthesis or replication which is 6-8 hours.[14] Microscopically most of these phases are not detectable except the M phase and even in the M phase mitosis is only detectable in metaphase and anaphase. Hence, the major part of the cell proliferative activity is missed by microscopy. These can only be detected by Immunohistochemistry such as Ki67 & PCNA or titrated thymidine.[15]

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Thus, all the proliferation detected by these methods are much more in number and specific. This discordance between actual DNA synthesis activity and the mitotic count has also been reported by other workers.

This perhaps is the reason that all those methods which take mitosis count as a parameter to classify prognosticate a tumour is not very accurate and widely accepted. However, Nunna et al. found that mitotic activity index was higher in patients having better survival chances and low recurrence rates. The research study mentioned the lack of significant association of the Mitotic Activity Index in relation to the types of differentiation could be due to a tumour having a migratory phenotype rather than a proliferative phenotype as can be seen in late-stage tumours.[16]

Hence, we propose that all such methods of detecting tumour type and prognostication should be supplemented with Ki67 or other immunohistochemistry markers as has been done in luminal classification of breast carcinoma.

**Conflict of Interest:** None

## References

1. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol. 2015;8(9):11884-94. [Google Scholar]
2. Khandekar SP, Bagdey PS, Tiwari RR. Oral cancer and some epidemiological factors: A hospital based study. Indian J Community Med. 2006;31(3):157-9. [Google Scholar]
3. Speight PM, Farthing PM, Bouquot JE. The pathology of oral cancer and precancer. Curr Diagn Pathol. 1996;3:165–76.[Google Scholar]
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68:394-424. [PubMed] [Google Scholar]
5. Saran R, Tiwari RK, Reddy PP, Ahuja YR. Risk assessment of oral cancer in patients with pre-cancerous states of the oral cavity using micronucleus test and challenge assay. Oral Oncol. 2008 Apr;44(4):354-60. [PubMed] [Google Scholar]
6. Liu X, Gao XL, Liang XH, Tang YL. The etiological spectrum of head and neck squamous cell carcinoma in young patients. Oncotarget. 2016 Oct;7(40):66226-38. [PubMed] [Google Scholar]
7. Dhanuthai K, Rojanawatsirivej S, Thosaporn W, Kintarak S, Subarnbhesaj A, Darling M, Kryshtalskyj E, Chiang CP, Shin HI, Choi SY, Lee SS, Aminishakib P. Oral cancer: A multicenter study. Med Oral Patol Oral Cir Bucal. 2018 Jan;23(1):e23-9. [PubMed] [Google Scholar]
8. Subashini V. Mitotic figures evaluation in oral squamous cell carcinoma using crystal violet and feulgen stains - A comparative study. Int J Orofac Bio. 2019 Oct;3(2):42-4. [Google Scholar]
9. Haschek WM, Rousseaux CG, Wallig MA, Bolon B, Ochoa R, editors. Haschek and Rousseaux's handbook of toxicologic pathology. Academic Press; 2013 May 1. [Google Scholar]
10. Shah S, Kaur M. Biomarkers and chemopreventives in oral carcinogenesis and its prevention. J Oral Maxillofac Pathol. 2014 Jan;18(1):69-76. [PubMed] [Google Scholar]
11. Meyer JS, Alvarez C, Milikowski C, Olson N, Russo I, Russo J, Glass A, Zehnbauer BA, Lister K, Parwaresch R; Cooperative Breast Cancer Tissue Resource. Breast carcinoma malignancy grading by Bloom-Richardson system vs proliferation index: reproducibility of grade and advantages of proliferation index. Mod Pathol. 2005 Aug;18(8):1067-78. [PubMed] [Google Scholar]
12. Kapoor K, Puri A, Prakash A, Jazib Sharma G. Mitotic counting and its significance in histopathological grading of OSCC & oral epithelial dysplasia. Head talk. 2013;5(6):35-7.
13. Kamel OW, Franklin WA, Ringus JC, Meyer JS. Thymidine labeling index and Ki-67 growth fraction in lesions of the breast. Am J Pathol. 1989 Jan;134(1):107-13. [PubMed] [Google Scholar]
14. Cooper GM. The eukaryotic cell cycle. The cell: a molecular approach. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. [Google Scholar]
15. Bologna-Molina, Ronell et al. Comparison of the value of PCNA and Ki-67 as markers of cell proliferation in ameloblastic tumors. Med Oral Patol Oral Cir Bucal. 2013 Mar;18(2):e174-9. [PubMed] [Google Scholar]
16. Chitra NS, Boaz K, Srikant N, Lewis AJ, Sneha KS. Pattern-Corrected Mitotic Activity Index (PMAI): A novel prognosticator of oral squamous cell carcinoma. Turk Patoloji Derg. 2020;36(1):31-8. [PubMed] [Google Scholar]