Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

To Assess the Interobserver Variability in Mitotic Figure Counting in Various Grades of Oral Squamous Cell Carcinoma

Afreen Nadaf[1], Rezhat Abbas[2*], Suheel Hamid Latoo[3]

[1]Assistant Professor, Department of Oral Pathology & Microbiology, Govt. Dental College & Hospital Srinagar, Jammu and Kashmir, India.
Email: aanadaf@gmail.com
Orcid ID: 0009-0004-6601-3872
[*2]Postgraduate Scholar, Department of Oral Pathology & Microbiology, Govt. Dental College & Hospital Srinagar, Jammu and Kashmir, India.
Email: writetoempire@gmail.com,
Orcid ID: 0000-0003-1413-8337
[3]Professor & Head, Department of Oral Pathology & Microbiology, Govt. Dental College & Hospital Srinagar, Jammu and Kashmir, India.
Email: suhaillatoo@gmail.com
Orcid ID: 000-0003-3655-3363

*Corresponding author

Received: 05 December 2022
Revised: 11 January 2023
Accepted: 26 January 2023
Published: 28 February 2023

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80–90% of all malignant neoplasms of the oral cavity. The increasing cases of oral cancer are the most important concern for community health. Defects of mitosis result in various nuclear abnormalities namely micronuclei, binucleation, broken egg appearance, pyknotic nuclei, and increased numbers of mitotic figures. The rationale for mitotic counting is that it is frequently used for classification and grading of tumors, prediction of prognosis of tumors and even advocated as a decision point for treatment. Reproducibility of the mitotic counting is paramount for the assessment of malignancy on a histologic scale. Considering this, we decided to assess the inter-observer variability in mitotic figure counting in various grades of oral squamous cell carcinoma. **Material & Methods:** A retrospective study was carried out on 48 formalin fixed paraffin embedded tissue blocks of the confirmed cases of Oral squamous cell carcinoma in the archives of department of oral pathology, Govt. Dental College & Hospital, Srinagar. Mitotic figure counting was done by two independent, mutually blind observers. The data was assessed for inter-observer variability in counting of figures. **Results:** Least difference was seen in cases of WDSCC up to the maximum difference of 3 mitoses. MDSCC showed modest differences in observations, with a maximum difference of 4 mitoses. PDSCC cases had the highest inter-observer variability, with a maximum difference of 5 mitoses. **Conclusion:** Mitosis counting has been shown most convincingly to provide independent prognostic value and is the most well established component of the histological grading systems of OSCC. Reproducibility of the mitotic counting is paramount for the assessment of malignancy on a histologic scale.

Keywords:- Oral squamous cell carcinoma, mitotic figure, interobserver variability..

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common oral malignancy, representing up to 80–90% of all malignant neoplasms of the oral cavity.[1] It is defined as "A malignant epithelial neoplasm exhibiting squamous differentiation as characterized by the formation of keratin and or the presence of intercellular bridges" (Pindborg J J. et al, 1997).[2] Globally, oral cancer ranks sixth among all types of cancer. India has the largest number of oral cancer cases and one-third of the total burden of oral cancer globally. The increasing cases of oral cancer are the most important concern for community health as it is one of the common types of cancers in India.[3]

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

Cell division occurs in defined stages, which together comprise the cell cycle. There are two types of cell division: Meiosis and Mitosis. Mitosis is conventionally divided into 5 phases, which include prophase, prometaphase, metaphase, anaphase and telophase.[4] Defects of mitosis result in various nuclear abnormalities namely micronuclei, binucleation, broken egg appearance, pyknotic nuclei, and increased numbers of mitotic figures. These mitotic figures (MFs) are commonly seen in oral epithelial dysplasia and squamous cell carcinoma. Location and increased numbers of mitotic figures are important criteria that carry increased weightage in the grading of dysplasias & OSCC's.[5] The rationale for mitotic counting is that it is frequently used for classification and grading of tumors, prediction of prognosis of tumors and even advocated as a decision point for treatment. Reproducibility of the mitotic counting is paramount for the assessment of malignancy on a histologic scale.[6]

MATERIAL AND METHODS

A retrospective study was carried out on 48 formalin fixed paraffin embedded tissue blocks of the confirmed cases of Oral squamous cell carcinoma in the archives of department of oral pathology, Govt. Dental College & Hospital, Srinagar. Mitotic figure counting was done by two independent, mutually blind observers as per the guidelines proposed by Baak and Oort (1983),[6] using an ordinary light microscope with 10X ocular and a 40X objective. The data was assessed for inter-observer variability in counting of figures.

RESULTS

The mitotic counts of 48 cases of oral squamous cell carcinoma that were classified into well-differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma respectively and estimated by 2 independent observers are presented in [Tables 1, 2 and Figure 1-3]. In cases of WDSCC, no difference in mitosis was found in 2 cases; a difference of 2 mitoses was observed in 10 cases; and a difference of 3 mitoses was found in 4 cases. In MDSCC cases, 1 case didn't show any difference in observations, a difference of 1 mitosis was seen in 4 cases, a difference of 2 mitoses was found in 5 cases, a difference of 3 mitoses was seen in 4 cases, and a difference of 4 mitoses was found in 2 cases. In cases of PDSCC, 4 cases showed a difference of 1 mitosis, and 6 cases showed a difference of 2 mitoses. A difference of 3 mitoses was seen in 4 cases, a difference of 4 mitoses was seen in 1 case, and 1 case showed a difference of 5 mitoses.

The least difference was seen in cases of WDSCC up to the maximum difference of 3 mitoses. MDSCC showed modest differences in observations, with a maximum difference of 4 mitoses. PDSCC cases had the highest inter-observer variability, with a maximum difference of 5 mitoses. Nevertheless, the differences were not statistically significant [Table 3].

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

Table 1: Inter-observer variability in mitotic figure counting (MF/10hpf) in different grades of oral squamous cell carcinoma, (n=48).

WDSCC (n=16)			**MDSCC(n=16)**		**PDSCC(n=16)**	
Case no	**Observer I**	**Observer II**	**Observer I**	**Observer II**	**Observer I**	**Observer II**
1.	04	03	06	08	08	06
2.	10	09	12	10	21	18
3.	08	10	10	13	20	19
4.	12	13	15	16	18	15
5.	07	06	16	12	19	21
6.	08	09	14	13	24	20
7.	09	10	18	17	23	22
8.	10	08	13	10	25	20
9.	11	09	18	15	26	24
10.	12	10	13	13	22	21
11.	10	11	14	10	23	20
12.	09	10	13	11	24	21
13.	13	14	15	13	20	22
14.	11	10	16	15	19	17
15.	12	12	14	12	18	16
16.	13	13	13	10	17	18

Table 2: The difference in average counts per 10 HPFs between the 2 pathologists in different grades of OSCC.

S.no	**Difference in counting of mitotic figures**	**WDSCC**	**MDSCC**	**PDSCC**
1.	No difference in mitosis	2 cases	1 case	---------
2.	Difference of 1 mitosis was observed	10 cases	4 cases	4 cases
3.	Difference of 2 mitosis was observed	4 cases	5 cases	6 cases
4.	Difference of 3 mitosis was observed	-------	4 cases	4 cases
5.	Difference of 4 mitosis was observed	-------	2 cases	1 case
6.	Difference of 5 mitosis was observed	--------	---------	1 case

Table 3: Statistical analysis of the observations by two independent observers

WDSCC		**MDSCC**		**PDSCC**	
Observer I	**Observer II**	**Observer I**	**Observer II**	**Observer I**	**Observer II**
Mean= 9.93	Mean=9.93	Mean=13.75	Mean=12.37	Mean=20.43	Mean= 18.75
SD= 2.40	SD=2.40	SD=2.93	SD=2.50	SD=4.28	SD= 4.13
P=0.5		P=0.08		P=0.13	

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

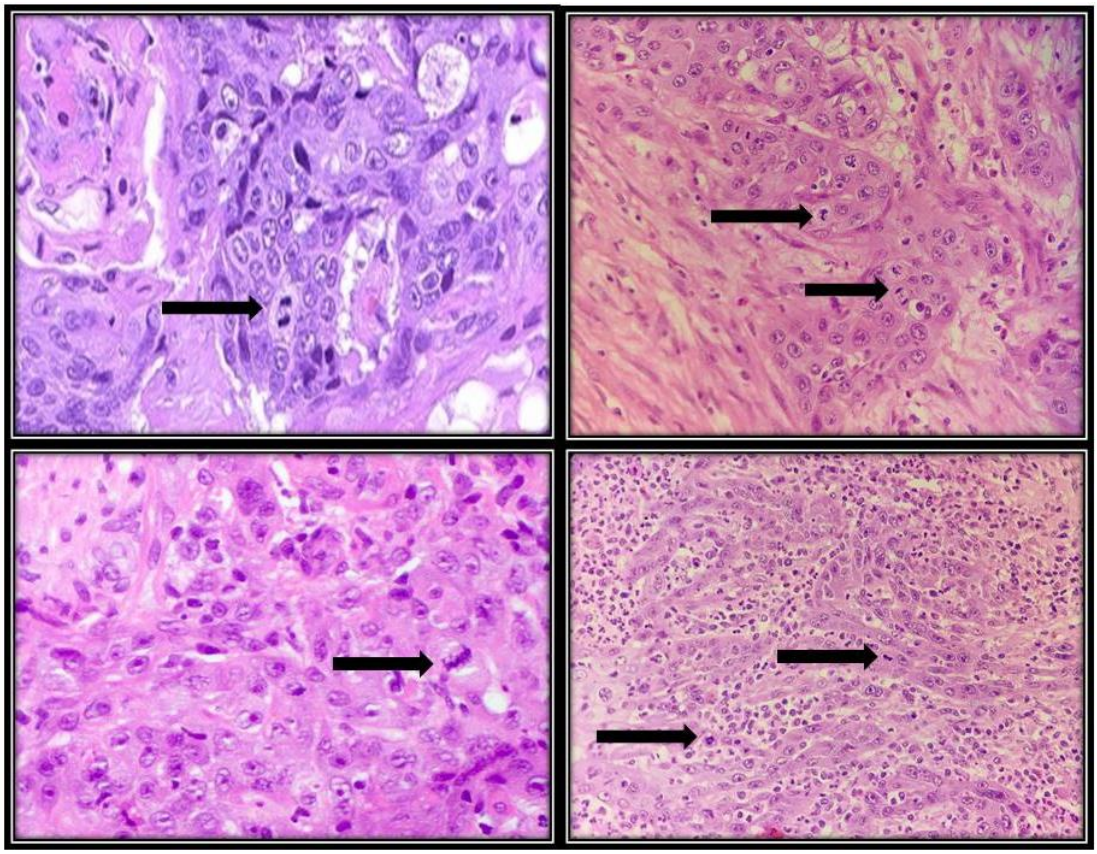
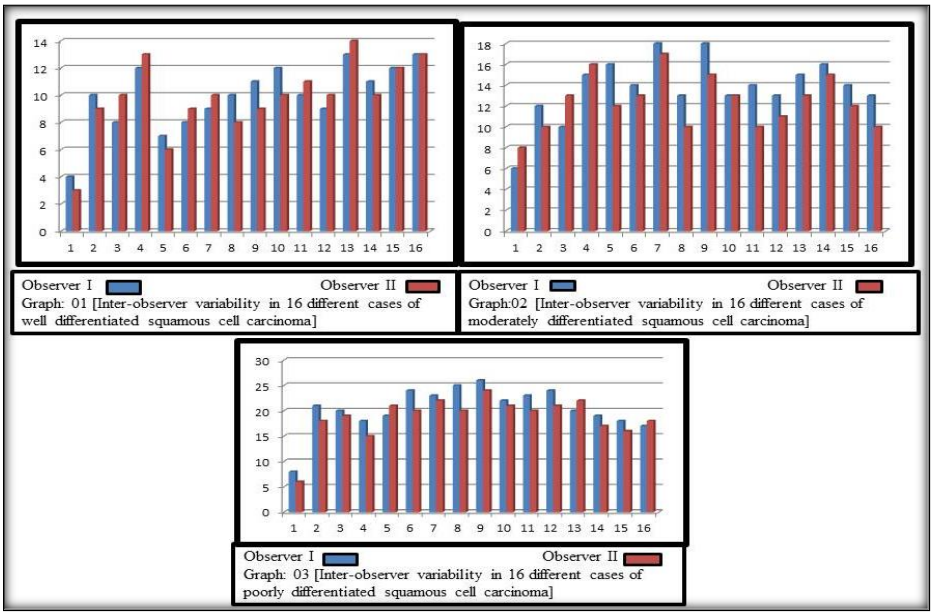


Figure 1: Histopathological photomicrographs depicting mitotic figures.



Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

DISCUSSION

There is an increasing awareness that proliferation is one of the most important features characterizing the malignant phenotype. In general, a high proliferation rate is found in malignant tumors and only rarely in benign tumors. Furthermore, in malignant tumors high proliferation rates are associated with a worse prognosis and poorer response to radiotherapy or chemotherapy than neoplasms with low rates.[7] The mitotic count is the most commonly used method of assessing the proliferative activity of a tumor. Although a number of other approaches exist, such as immunohistochemistry using different antibodies such as Ki 67, PCNA, or topoisomerase II, the mitotic count has the advantage that it can be performed in routine haematoxylin and eosin (H&E)-stained slides. For this reason the mitotic count or mitotic index (MI) is frequently used for classification, grading, predicting prognosis of tumors and sometimes even advocated as a decision point for treatment. Reproducibility of the mitotic counting is paramount for the assessment of malignancy on a histologic scale.[8]

The aim of this study was to evaluate the inter-observer variations in counting of mitotic figures in different grades of oral squamous cell carcinoma.

In our study, least difference was seen in cases of WDSCC up to the maximum difference of 3 mitoses. MDSCC showed modest differences in observations, with a maximum difference of 4 mitoses. PDSCC cases had the highest inter-observer variability, with a maximum difference of 5 mitoses.

Similar findings were observed by Yadav KS et al (2012).[6] The differences in mitosis in their study (n=30) were as follows: In cases of WDSCC, there was no difference in mitoses in 1 case; in 4 cases, a difference of 1 mitosis was observed; in 3 cases, a difference of 2 mitoses was observed; and in 2 cases, a difference of 3 mitoses was observed. In cases of MDSCC, there was no difference in mitosis in 4 cases; a difference of 1 mitosis was seen in 4 cases; a difference of 2 mitoses was seen in 2 cases. In cases of PDSCC, no difference was seen in 3 cases; a difference of 1 mitosis was seen in 3 cases; a difference of 2 mitoses was seen in 2 cases; and a difference of 3 mitoses was seen in 2 cases. In our study highest difference was seen in PDSCC (difference of 5 mitosis) cases and least difference in WDSCC cases.[6]

There has been considerable controversy in establishing mitotic figure as diagnostic and prognostic criteria in tumors. Steven G. Silverberg believes that it cannot be used as the sole criterion for distinguishing benign from malignant tumors because of its poor reproducibility & time constraints.[9] A further criticism of mitotic index as a measure of proliferation is that the duration of the mitotic phase of the cell cycle is variable, and hence the correlation of number of mitoses and proliferation rate is not necessarily linear.[10]

According to Yadav KS6, the lack of reproducibility in counting is largely due to the lack of strict counting protocols. In their study, the counting was done using a strict protocol, and the results were highly reproducible. They believe that the criticism that mitotic counting requires extra time is unfounded, and the procedure is certainly justified considering that mitotic figure count has a strong prognostic

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

value and is a significant parameter in the selection of treatment for a patient.[6]

Clayton reported a study of 378 node-negative breast cancers and found that, on multivariate analysis, mitotic count was a stronger predictor of survival than tumor size, lymphatic invasion or skin invasion. Patients with more than 4.5 mitotic figures per 10 HPFs had a 2.8-fold increase in the risk of death.[11]

The scoring of mitotic index does seem to be relatively consistent in routine practice in a study by van Diest and colleagues. However, discrepancies are inevitable. The reasons for the discrepancies are mainly due to poor tissue processing, inaccurate counting or failure to follow the guidelines for selection of the counting area. There might be difficulties to identify mitotic cells due to confusion with apoptosis or nuclear pyknosis.[12] Silverberg (1976) and Stenkvist (1979) obtained extremely variable counts in mitosis counting which can be attributed to the fact that the investigators were not provided with any criteria/ guidelines before the evaluation of slides.[9,13] Ellison et al (1987) in their study demonstrated that in 91% of patients the difference in average number of mitotic figure/ HPFs was less than 2 mitoses, thereby elaborating the fact that mitotic figures can be recognized readily with a high degree of consistency.[14]

CONCLUSIONS

Proliferation plays an important role in the clinical behaviour of oral squamous cell carcinoma. Increased proliferation correlates strongly with poor prognosis. Out of the different methods to assess proliferation, mitosis counting has been shown most convincingly to provide independent prognostic value and is the most well established component of the histological grading systems of OSCC.

REFERENCES

1. Pires FR, Ramos AB, Oliveira JB, Tavares AS, Luz PS, Santos TC. Oral squamous cell carcinoma: clinicopathological features from 346 cases from a single oral pathology service during an 8-year period. J Appl Oral Sci. 2013;21(5):460-7. doi: 10.1590/1679-775720130317.
2. Rivera C, Venegas B. Histological and molecular aspects of oral squamous cell carcinoma (Review). Oncol Lett. 2014;8(1):7-11. doi: 10.3892/ol.2014.2103.
3. Borse V, Konwar AN, Buragohain P. Oral cancer diagnosis and perspectives in India. Sens Int. 2020;1:100046. doi: 10.1016/j.sintl.2020.100046.
4. Thavarool SB, Muttath G, Nayanar S, Duraisamy K, Bhat P, Shringarpure K, et al. Improved survival among oral cancer patients: findings from a retrospective study at a tertiary care cancer centre in rural Kerala, India. World J Surg Oncol. 2019;17(1):15. doi: 10.1186/s12957-018-1550-z.
5. Bavle RM. Mitosis at a glance. J Oral Maxillofac Pathol. 2014;18(Suppl 1):S2-5. doi: 10.4103/0973-029X.141175.
6. Yadav KS, Gonuguntla S, Ealla KKR, Velidandla SR, Reddy CRC, Prasanna MD, et al. Assessment of Interobserver Variability in Mitotic Figure Counting in Different Histological Grades of Oral Squamous Cell Carcinoma. J Contemp Dent Pract. 2012;13(3):339-344.
7. Baak JP. Mitosis counting in tumors. Hum Pathol. 1990;21(7):683-5. doi: 10.1016/0046-8177(90)90026-2.
8. Thunnissen FB, Ambergen AW, Koss M, Travis WD, O'Leary TJ, Ellis IO. Mitotic counting in surgical pathology: sampling bias, heterogeneity and statistical uncertainty. Histopathology. 2001;39(1):1-8. doi: 10.1046/j.1365-2559.2001.01187.x.
9. Silverberg SG. Reproducibility of the mitosis count in the histologic diagnosis of smooth muscle tumors of the uterus. Hum Pathol. 1976;7(4):451-4. doi: 10.1016/s0046-8177(76)80058-5.

Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-9, Issue-2 | March- April 2023
DOI: 10.53339/aimdr.2023.9.2.5
Page no- 24-30 | Section- Research Article (Oral Pathology & Microbiology)

10. Beresford MJ, Wilson GD, Makris A. Measuring proliferation in breast cancer: practicalities and applications. Breast Cancer Res. 2006;8(6):216. doi: 10.1186/bcr1618.
11. Clayton F. Pathologic correlates of survival in 378 lymph node-negative infiltrating ductal breast carcinomas. Mitotic count is the best single predictor. Cancer. 1991; 68:1309–1317.
12. van Diest PJ, Baak JP, Matze-Cok P, Wisse-Brekelmans EC, van Galen CM, Kurver PH, et al. Reproducibility of mitosis counting in 2,469 breast cancer specimens: results from the Multicenter Morphometric Mammary Carcinoma Project. Hum Pathol. 1992;23(6):603-7. doi: 10.1016/0046-8177(92)90313-r.
13. Stenkvist B, Westman-Naeser S, Vegelius J, Holmquist J, Nordin B, Bengtsson E, et al. Analysis of reproducibility of subjective grading systems for breast carcinoma. J Clin Pathol. 1979;32(10):979-85. doi: 10.1136/jcp.32.10.979.
14. Ellison DJ, Nathwani BN, Metter GE, Turner RR, Burke JS, Miller TP, et al. Mitotic counts in follicular lymphomas. Hum Pathol. 1987;18(5):502-5. doi: 10.1016/s0046-8177(87)80035-7.

Source of Support: Nil, Conflict of Interest: None declare