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Original Article

Integrating Mcm-2 and Ki-67 immunohistochemistry with clinico-pathologic parameters for enhanced prognostic accuracy in oral verrucous lesions

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ABSTRACT

Background: Oral verrucous lesions (OVLs) present a diagnostic challenge due to their diverse and often confusing histopathological features. Accurate differentiation is essential for improving diagnosis and predicting prognosis. In addition to assessing overall survival (OS) and disease-free survival (DFS) in verrucous squamous cell carcinoma (VSCC) and conventional OSCC, this study seeks to evaluate the expression of Mcm-2 and Ki-67 in verrucous lesions and oral squamous cell carcinoma (OSCC). These findings will be correlated with the nuclear expression of Mcm-2 and Ki-67.

Methodology: Ninety tissue samples that were paraffin embedded and formalin-fixed were examined using immunohistochemistry to determine the expression of Mcm-2 and Ki-67. Data on survival and clinico-pathologic characteristics were taken from patient records. Statistical analyses were conducted using Independent T-tests, Cox regression models, and Kaplan-Meier survival analysis.

Results: Mcm-2 was identified as a more sensitive and prognostic marker compared to Ki-67 across the study groups. Mcm-2 overexpression was observed in all cases of verrucous hyperplasia with dysplasia, verrucous carcinoma (VC), VSCC, and conventional OSCC. The 3-year OS and DFS rates were lower in conventional OSCC (75 % and 64.3 %, respectively) compared to VSCC (90 % and 70 %).

Conclusion: This study represents the first initiative to employ both Mcm-2 and Ki-67 as proliferative markers for distinguishing between various oral verrucous lesions. Mcm-2 proves to be a valuable marker for differentiating between potentially malignant and malignant verrucous lesions. However, further validation with larger sample sizes and longer follow-up periods is necessary to confirm its role in predicting OS and DFS.

1. Introduction

The identification and classification of verrucous lesions manifesting orally present a diagnostic challenge for both clinicians and pathologists due to the heterogeneous nature and broad spectrum of differential diagnoses [1,2]. These lesions can be categorized as potentially malignant (PML) and malignant (ML), with a diverse collection of features. Thomas G J et al. (2009) have classified oral verrucous lesions as benign, PML, and ML. The distinction between oral verrucous hyperplasia (VH), a potentially malignant lesion, and oral verrucous carcinoma (OVC) can be clinically challenging due to their similar appearance. Shear and Pindborg conducted an in-depth histological examination aimed at differentiating between VH and VC, focusing on growth pattern, rete morphology, and the extent of cytological atypia [3].

The presence of less differentiated foci of invasive Oral Squamous Cell Carcinoma (OSCC) characterizes the Verrucous type of Squamous Cell Carcinoma (VSCC) or so-called 'hybrid verrucous carcinoma'. Differentiating between VCs and conventional OSCCs is imperative for better treatment and prognosis. Molecular approaches and immunohistochemistry, particularly the protein Mcm-2, show promise in providing valuable assistance in the study of these lesions. With strong prognostic potential, Mcm-2 can be a useful marker of the early identification of changed aberrant cells in lesions that may develop into cancer.

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The expression of MCM-2 and Ki-67 during the change from possibly malignant lesions to malignant verrucous lesions has not been thoroughly studied up to now. Furthermore, few studies have compared cell proliferation markers with Overall (OS) and Disease-free Survival (DFS) to determine prognosis in this migrating group of lesions. Thus, the current study aims to examine OS and DFS in oral possibly malignant & malignant verrucous lesions, as well as conventional squamous cell carcinoma of the mouth (OSCC), and to measure and compare the expression of Mcm-2 to the traditional proliferation marker Ki-67.

2. Methodology

Using immunohistochemistry (IHC), 90 cases (Normal Mucosa (NM), Verrucous Hyperplasia (VH) with or with no dysplasia, Verrucous Carcinoma (VC), Verrucous Squamous Cell Carcinoma (VSCC), and conventional Oral Squamous Cell Carcinoma (OSCC) were examined for Mcm-2 and Ki-67 expression between 2012 and 2016. The SDM College of Dental Sciences and Hospital's IRB in Dharwad provided ethical approval. Groups A, B, C, D, E, and F were designated as NM, VH without dysplasia, VH with dysplasia, VC, VSCC, and OSCC, respectively, for the purposes of the study. From patient files, clinical information and survival statistics were gathered.

2.1. Immunohistochemical analysis

IHC analysis for Mcm-2 (Biogenex) and Ki-67 (Pathnsitu) was carried out on 4 μm paraffin-embedded tissue sections, in accordance with manufacturer guidelines (BioGenex & Path *in-situ*). After being deparaffinized and incubated for an entire night at 37°C, the slides were subjected to pressure cooker antigen retrieval using tris-EDTA buffer. After treating the slides with power block and peroxide block, they were allowed to come to room temperature for an hour before being incubated with the primary antibodies for Ki-67 and Mcm-2. Following PBS washes, a super-enhancer was used, and the secondary antibody was then incubated for 30 min. To obtain colour intensity, 7–10 min were spent using DAB chromogen, and slides were then counterstained with Harris haematoxylin.

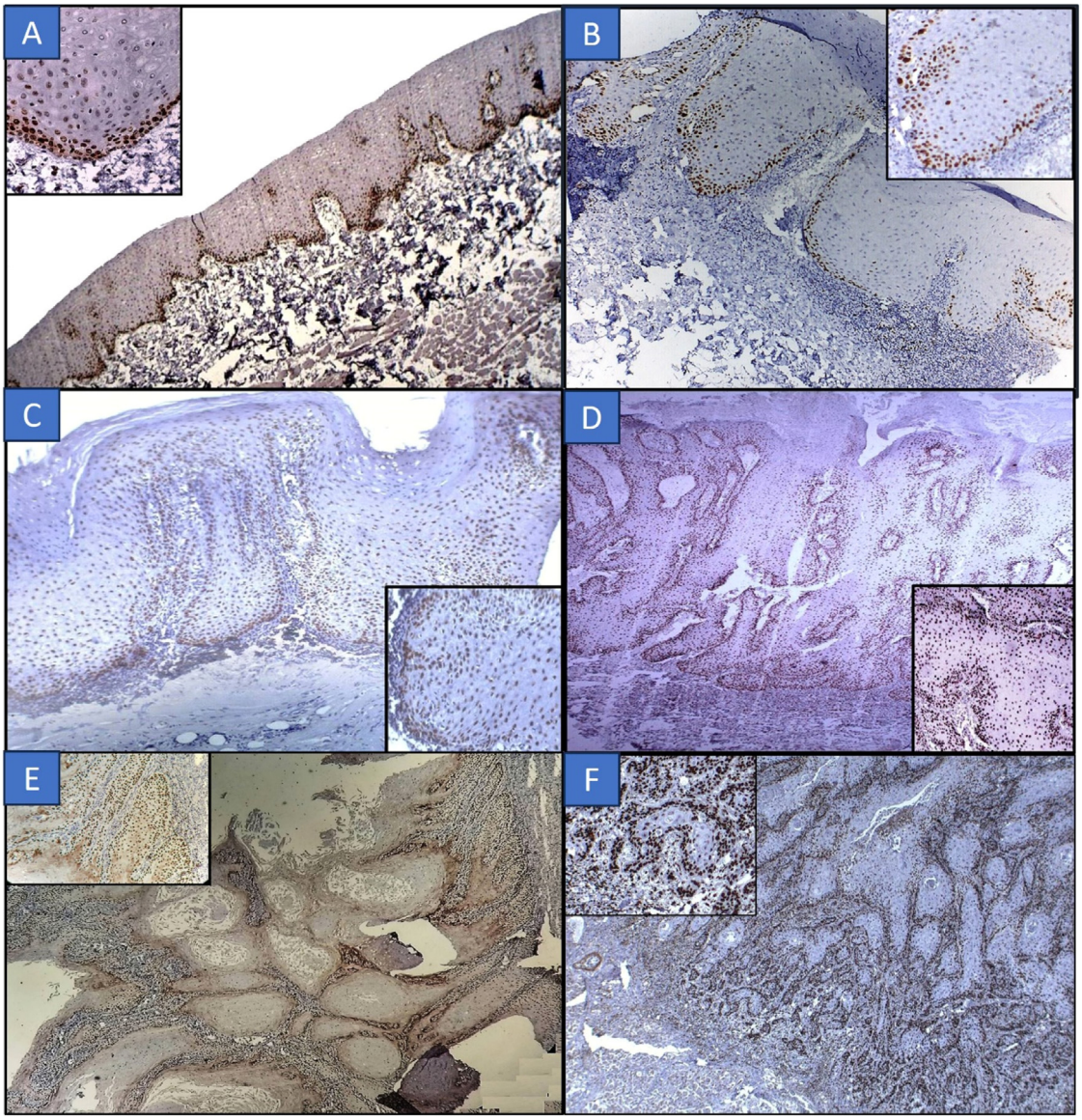

Fig. 1. MCM-2 staining shows nuclear expression in various epithelial layers: (A) basal/parabasal in NM epithelium; (B) basal/parabasal and some suprabasal cells in VH without dysplasia; (C) basal to >2/3rd of the epithelium in VH with dysplasia; (D) basal/parabasal and suprabasal cells in VC; (E) peripheral cells of tumor islands in VSCC; (F) peripheral cells in small groups of infiltrating tumor cells in conventional OSCC. (x10 and x40, IHC-Mo Ab Mcm-2).

2.2. Assessment of Mcm-2 and Ki-67 staining

A brown outcome at the target antigen location indicated positive staining. Two observers (A and B) quantitatively assessed the nuclear expressions of Mcm-2 and Ki-67 using the nuclear labelling index (nLI). Up to 500 tumour cells were counted per case using an eyeglass graticule set at 400x magnification for cell counting. The percentage of positive cells relative to all tumour cells was used to compute the nLI. The dependability between and among the observers was evaluated.

2.3. Statistical analysis

Inter-observer variability was assessed using Cohen's Kappa. T-tests compared the nLI of Mcm-2 and Ki-67 among groups A to F. Statistical analyses were conducted with SPSS version 20.0, with significance at $p < 0.05$. Cox-Regression model was used for multivariate analysis of clinico-pathologic parameters and Overall Survival (OS) in Groups E and F. Kaplan-Meier Survival Analysis examined 3-year OS and Disease-Free Survival (DFS) in relation to nLI of Mcm-2 and Ki-67 and treatment modality.

3. Results

Ninety participants in all were included in the current investigation, and the clinicopathological data were gathered from the case files. Nuclear expression of Mcm-2 & Ki-67 was positive in all samples of tissue from Groups A through F.

Variable nuclear expression of Mcm-2 and Ki-67 was observed in all groups (Figs. 1 and 2), and the mean labeling indices in each group are detailed in Table 1.

A noticeable increase in the nuclear staining of Mcm-2 and Ki-67 was observed across Groups A, B, C, D, E, and F. The mean nuclear labeling index of Mcm-2, compared to Ki-67, exhibited significant differences in Groups A, C, D, E, and F, with a higher quantitative nuclear expression of Mcm-2 than Ki-67.

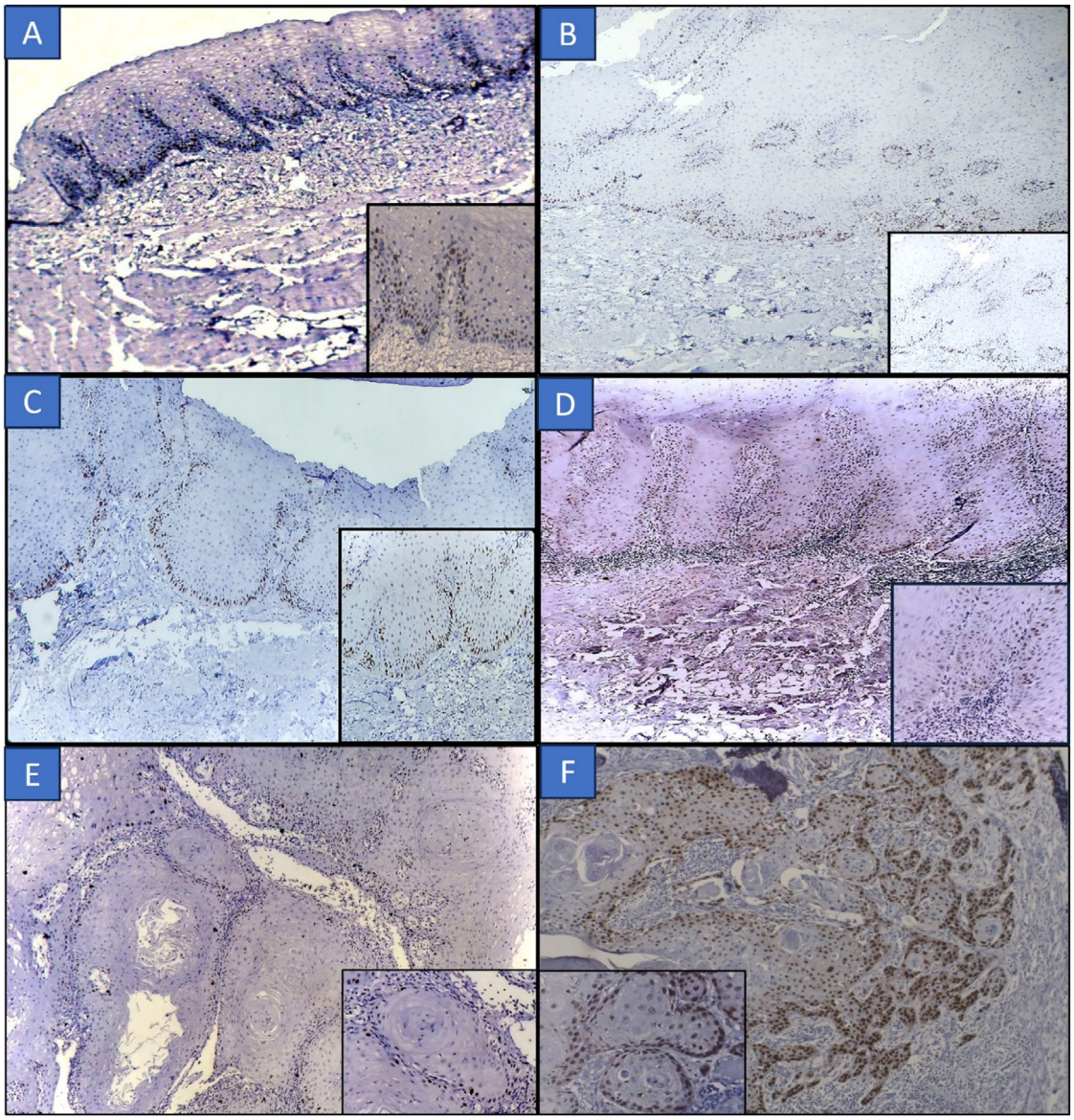

Fig. 2. KI-67 staining shows nuclear expression in various epithelial layers: (A) basal and suprabasal in NM epithelium; (B) basal/parabasal in VH without dysplasia; (C) basal/parabasal in VH with dysplasia; (D) basal, parabasal, and some suprabasal cells in VC; (E) peripheral cells of tumor islands in VSCC; (F) peripheral cells in infiltrating tumor islands in conventional OSCC. (x10 and x40, IHC-Mo Ab Ki-67).

Table 1
Comparison of Mcm-2 and Ki-67 mean nLI in NM with the other groups using the T-test.

Groups	Mcm-2	Ki-67	t value	Significance
NM (Group A)	49.08 ± 3.94	19.55 ± 2.96	18.905	<0.001*
VH without Dysplasia (Group B)	35.73±11.44	30.25±7.42	1.270	0.220 NS
VH with Dysplasia (Group C)	64.10±13.27	40.53±3.59	5.419	<0.001* HS
VC (Group D)	60.45±15.92	41.93±7.37	4.718	<0.001* HS
VSCC (Group E)	79.97±4.04	54.55±6.56	14.746	<0.001* HS
OSCC (Group F)	89.46±5.53	55.76±8.01	15.465	<0.001* HS

The current study involved conducting multivariate analysis using the Cox-Regression model to compare clinico-pathologic parameters and the 3-year Overall Survival in Groups E and F (Tables 2 and 3).

In Group E, VSCC, a statistically significant correlation was found between the overall survival and site affected, as cases with lesions on the buccal mucosa exhibited reduced overall survival ($p = 0.033$). Similarly, patients with habit association had decreased overall survival compared to those with no habit association, with statistical significance in tobacco consumption, betel quid chewing, and combination of 2 or more habits ($p < 0.05$). Cases with a depth of invasion (DOI) of 5–10 mm showed reduced overall survival in VSCC with statistical significance ($p = 0.038$). In Conventional OSCC, cases showing perineural invasion (PNI) and lymphovascular invasion (PVI) demonstrated reduced overall survival ($p < 0.05$).

A comparison of 3-year OS and DFS was made with the nuclear expression of Mcm-2 and Ki-67 in VSCC and OSCC (Tables 4 and 5).

The medians of both Mcm-2 and Ki-67 were evaluated and used as a cut-off value for comparing OS and DFS. In VSCC (Group E), it was observed that cases with a higher Mcm-2 index than the cut-off value had reduced OS and DFS, whereas cases below the cut-off value with lesser nuclear expression also had reduced OS and DFS (Figs. 3 and 4).

Similarly, VSCC cases with lower proliferative index of Ki-67 exhibited better OS and DFS. In conventional OSCC, although cases with higher Mcm-2 expression showed increased OS and DFS, a higher number of deaths were observed in cases with a higher proliferative index.

Table 2
Multi-variate analysis of clinico-pathologic parameters and overall survival in Group E (VSCC).

Parameter	Lower CI	Upper CI	DF	SIG.
Age	0.165	72.432	1	0.425 NS
Gender	0.043	23,270.114	1	0.306 NS
Site	2.791	6.002E	1	0.033 S
Habits				
None	–	–	0	–
Tobacco	6.359	3.779E17	1	0.032 S
Betel-quid	1.397	3.152E9	1	0.043 S
Combi	142.594	7.372E20	1	0.016 S
Tumour size				
T1	–	–	2	0.328 NS
T2	0.00	25.074	1	0.170 NS
T3	0.060	16.626	1	1.000 NS
Type of growth	0.000	5.167	1	0.170 NS
pTNM	–	–	0	–
PVI	–	–	0	–
PNI	–	–	0	–
ECS	–	–	0	–
DOI				
1–5 mm	–	–	2	0.099 NS
5–10 mm	0.000	0.514	1	0.038 S
> 10 mm	0.000	66.265	1	0.467 NS
POI				
Type1	–	–	2	0.434 NS
Type 2	0.056	2533.162	1	0.365 NS
Type 3	0.136	15,369.201	1	0.198 NS
Broder's grading	–	–	–	–

Table 3
Multi-variate analysis of clinico-pathologic parameters and overall survival in Group F (CONVENTIONAL OSCC).

Parameter	Lower CI	Upper CI	DF	SIG.
Age	0.00	6.579E8	1	0.462 NS
Gender	0.00	2.684E13	1	0.621 NS
Site	–	–	0	–
Habits				
None	–	–	1	0.881 NS
Tobacco	–	–	2	0.515 NS
Betel-quid	0.00	1.162E13	1	0.596 NS
Combi	0.00	4.891E11	1	0.926 NS
Tumour size				
T1	0.01	13.578	1	0.353 NS
T2	0.00	5.532E13	1	0.615 NS
T3	0.00	1.547E16	1	0.626 NS
Type of growth	0.00	3.746E	1	0.523 NS
pTNM				
Stage 1	–	–	2	0.305 NS
Stage 2	0.00	2.24E	1	0.867 NS
Stage 3	0.00	96.008	1	0.281 NS
PVI	4.909	1.216E111	1	0.026 S
PNI	0.00	1.354	1	0.05 S
ECS	0.00	2.485E8	1	0.850 NS
DOI				
1–5 mm	–	–	2	0.787 NS
5–10 mm	0.00	3.212E20	1	0.927 NS
> 10 mm	0.00	2,932,846.102	1	0.926 NS
POI				
Type1	0	–	2	0.235 NS
Type 2	0.00	201.531	1	0.148 NS
Type 3	0.00	12.974	1	0.096 NS
Broder's grading	–	–	1	0.078 NS

A comparison of the 3-year OS and DFS was carried out with treatment modalities in Groups E and F (Tables 6 and 7).

In VSCC (Group E), the OS and DFS were higher in patients treated with only surgery, whereas patients treated with a combination of surgery and radiotherapy had reduced OS & DFS ($p = 0.008$ & $p = 0.048$). In conventional OSCC, patients treated with a combination of surgery and radiotherapy exhibited higher OS and DFS compared to those treated with adjuvant radiotherapy along with surgery ($p = 0.655$ & $p = 0.632$).

The heat map and pair plots reveal a strong positive correlation between Mcm-2 and Ki-67 (0.88), indicating that higher levels of Mcm-2 are associated with higher Ki-67 levels, which aligns with their roles as proliferation markers. There is a moderate positive correlation between Mcm-2 and L-value (0.49), suggesting a potential relationship between Mcm-2 expression and the clinico-pathologic parameter represented by L-value. The correlation between Ki-67 and L-value is weak (0.001), indicating little to no relationship. These findings suggest that integrating Mcm-2 and Ki-67 immunohistochemistry with clinico-pathologic parameters like L-value could enhance prognostic accuracy in oral verrucous lesions (Fig. 5).

The bar plot and distribution plots indicate that Mcm-2 and Ki-67 are more highly expressed in more severe clinical groups (e.g., VC and OSCC), which can be used to differentiate between varying levels of disease severity. The box plots reveal that within specific groups, there are variations in the expression of variables A, B, and C, which could reflect underlying biological differences. Integrating these immunohistochemical markers with clinico-pathologic parameters enhances the prognostic accuracy for oral verrucous lesions by identifying significant expression patterns and variability across different clinical presentations (Fig. 6)

4. Discussion

Oral squamous cell carcinoma (OSCC) remains significant, constituting over 90 % of all oral malignancies. Verrucous carcinoma (VC), a

Table 4
Comparison between overall survival/ disease free survival and Mcm-2/Ki-67 nLI in group E (VSCC).

SURVIVAL	MARKER	SURVIVAL ESTIMATE	Std. Error	Lower CI	Upper CI	Log-Rank (Mantel-Cox)		
Marker	Mcm-2					Chi-Sq	df	Sig.
3 YR OS	< cut-off	49.600	2.783	44.146	55.054	0.278	1	0.578
	> cut-off	49.600	2.277	45.137	54.063			
3 YR DFS	< cut-off	44.800	3.580	37.783	51.817	.537	1	0.464
	> cut-off	46.800	3.301	40.330	53.270			
Marker	Ki-67					Chi-Sq	df	Sig.
3 YR OS	< cut-off	40.889	2.017	36.936	44.842	.117	1	0.732
	> cut-off	40.875	2.503	35.969	45.781			
3 YR DFS	< cut-off	41.857	2.483	36.991	46.723	.132	1	0.716
	> cut-off	41.571	2.776	36.130	47.013			

Table 5
Comparison between Overall Survival and Disease-Free Survival & Mcm-2/Ki-67 nLI in Group F (Conventional OSCC).

SURVIVAL	MARKER	SURVIVAL ESTIMATE	SE	L CI	U CI	Log-Rank (Mantel-Cox)		
Marker	Mcm-2					Chi-Sq	df	Sig.
3 YR OS	< cut-off	39.200	.727	37.775	40.625	.445	1	0.504
	> cut-off	40.000	1.239	37.571	42.429			
3 YR DFS	< cut-off	39.852	1.068	37.758	41.945	.639	1	0.424
	> cut-off	41.000	1.317	38.420	43.580			
Marker	Ki-67					Chi-Sq	df	Sig.
3 YR OS	< cut-off	38.286	.565	37.177	39.394	3.650	1	0.05*
	> cut-off	40.545	.908	38.765	42.325			
3 YR DFS	< cut-off	38.000	.456	37.105	38.895	5.511	1	.019*
	> cut-off	41.519	1.063	39.435	43.602			

distinct subtype of OSCC, displays unique clinicopathologic characteristics and a relatively slow progression [4,5]. Researchers have proposed a variant termed verrucous squamous cell carcinoma (VSCC), representing a blend of OSCC and VC [6,7]. However, limited studies have explored this variant thus far [5,8]. Despite advances in diagnostic and therapeutic approaches, there has been an upward trend in the overall mortality of OSCC patients [9]. Hence, it is imperative to differentiate between the slow-growing VC and the aggressive VSCC, which has metastatic potential and high mortality risk, and to compare these conditions with conventional OSCC [10,11]. Identifying potential cell proliferation markers that could assist in predicting patient prognosis and survival is crucial. Using the cell proliferative marker Mcm-2 and Ki-67, the current study's main goal was to distinguish between different kinds of lesions thought to be a part of a range of clinico-histopathologic disorders. A comparison of the overall and disease-free rate of survival of individuals with VSCC and OSCC was another goal of the study.

In the investigation, verrucous hyperplasia (VH) was classified into two groups: VH without dysplasia (Group B) and lesions showing atypia or dysplasia (Group C). In Group B, there were a few cells in the middle third of the epithelium and Mcm-2 and Ki-67 expression was seen in the basal and suprabasal layers. This indicates that VH cells divide at a controlled rate without dysplasia, with the majority of the outermost layers' cells displaying differentiation. In PVL, Gouvea and colleagues (2010) found that Mcm-2 and Mcm-5 levels of expression were higher than Ki67 [12]. Similar findings were reported as Niranjan et al. (2018) in a prior study [13]. Mcm-2 expression of oral verrucous hyperplasia has been the subject of a few investigations [5,12,13]. To make firm findings, more research with a bigger sample size is required.

With the exception of the differentiated cells in the superficial layers, Mcm-2 nuclear expression was seen in the basal, parabasal, and middle third layers of the epithelium in Group C (VH with Dysplasia). With a nuclear labelling index (nLI) of 64.10 percent, dysplastic cells were consistently re-entering the cell cycle. According to Torres-Rendon et al. [9], who examined Mcm-2 expression in oral epithelial dysplasia and normal mucosa, dysplasia had higher nuclear expression (73.62 %) because of continuous cell-cycle re-entry. This conclusion is consistent with the current investigation. Group C's nLI of 40.53 % indicated that ki-67 expression was restricted to the basal and suprabasal layers. Oral Verrucous Hyperplasia (OVH) with or without dysplasia and Oral Verrucous Carcinoma (OVC) were explored by Klieb et al. (2009). They found nuclear expression in the basal and suprabasal layers, with higher expression.

For Group D (VC), Mcm-2 nuclear expression was observed in the basal to superficial layers, covering over two-thirds of the epithelium (nLI-60.45 %). The expression was slightly lower than in Group C (VH with dysplasia), which may be due to fewer atypical or dysplastic characteristics in VC [14]. Gimenez Conti et al. (1996) described VC as highly differentiated with minimal mitotic activity, so only cells in the cell cycle or the least differentiated cells exhibit Mcm-2 expression [15]. Ki-67 expression in Group D was found in the basal and suprabasal layers, with few cells in the middle third (nLI-41.93 %). Similar findings were reported by Zargaran et al. (2012) [14], Saito et al. (1999) [16], Adegboyega et al. (2005) [17], and Theegarten et al. (1997) [18].

In Group E (VSCC), Mcm-2 nuclear expression was observed from the basal to superficial layers at the tumor margin, excluding the most superficial differentiated cells. Expression was also noted in the tumor islands of the invasive tumor front (ITF), primarily in peripheral cells. The mean nLI was 79.97 %. Ki-67 expression was observed in both the tumor margin and tumor islands, limited to the periphery. The mean nLI was 54.55 %. The study indicates higher Mcm-2 expression in VSCC compared to Groups B, C, and D, suggesting continuous proliferation in VSCC cells. No existing studies on VSCC using Mcm-2 and Ki-67 markers necessitate further research.

In Group F (Conventional OSCC), Mcm-2 exhibited the highest nuclear expression among all groups, with intense staining across nearly all epithelial layers, indicating a high proliferation state. When NM, VH, VC, and VSCC's nLI was compared to that of OSCC, statistically significant findings were observed. The highest Mcm-2

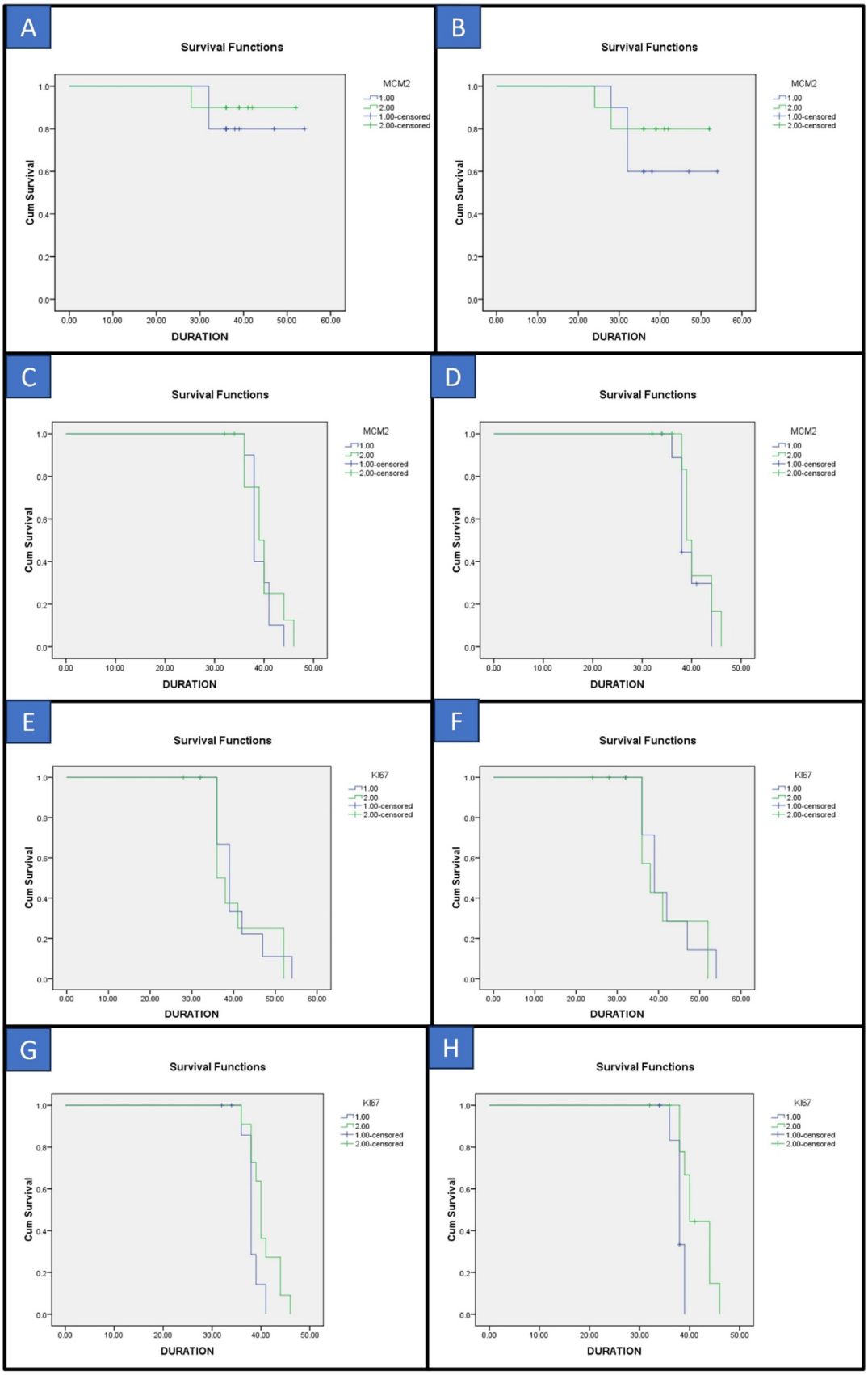

Fig. 3. Kaplan-Meier plots illustrating reduced 3-year OS and DFS for higher Mcm-2 and Ki-67 immunoexpression groups in VSCC (A, B, E, F) and conventional OSCC (C, D, G, H) across 20 cases each (Group E and F, 1 = <cut-off value, 2 = >cut-off value).

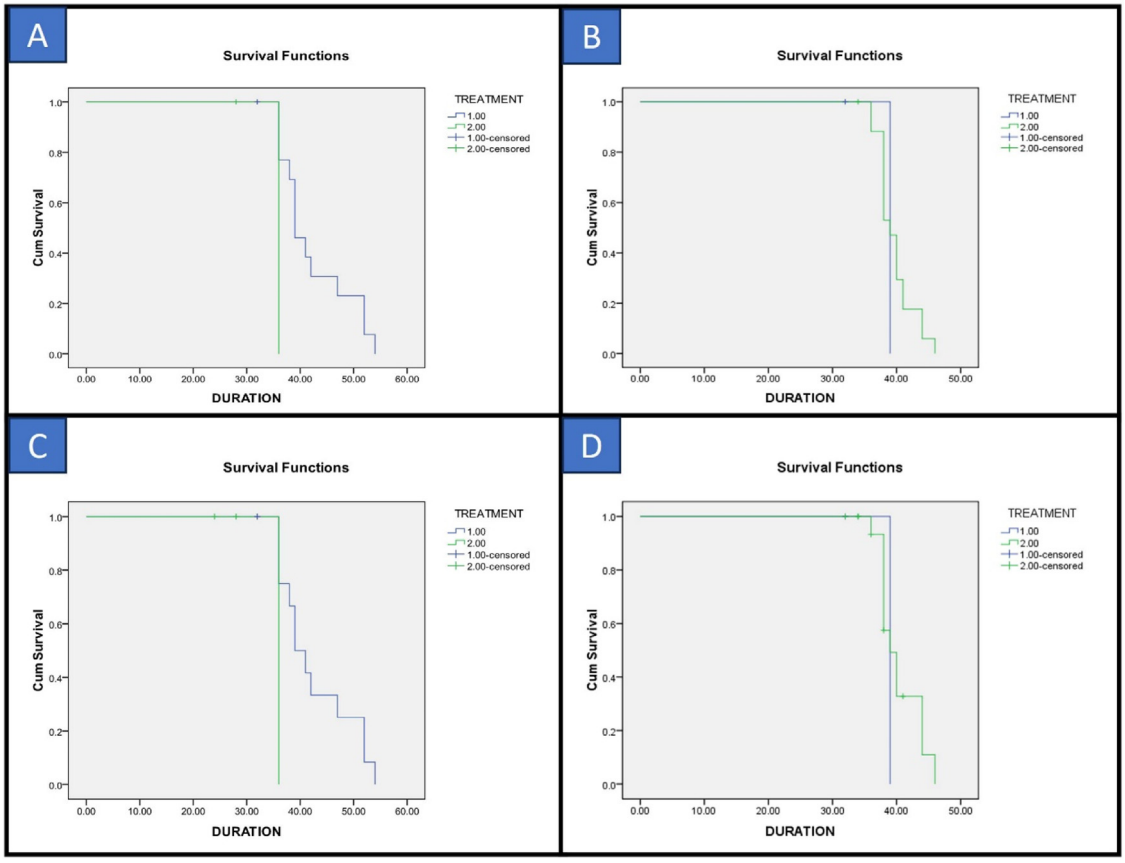

Fig. 4. A and B: Kaplan-Meier plots show reduced 3-year OS and DFS in patients with Group E (VSCC) treated with surgery and radiotherapy compared to surgery alone. C and D: Kaplan-Meier plots indicate reduced 3-year OS and DFS in patients with Group F (conventional OSCC) treated with surgery alone compared to combined treatment.

expression was found in OSCC in investigations conducted by Torres-Rendon et al. (2009) [9], Shalash (2012) [19], & Razavi and colleagues (2015) [20], which is in line with the current study's 89.22 % finding. Furthermore, compared to the tumour centre (TC) and tumour margin (TM), Alrani and colleagues [21] observed increased MCM-2 expression in the invasive tumour front (ITF).

Ki-67 expression was highest in Group E (55.76 %), observed in all cell layers from basal to superficial in the TM, and confined to peripheral cells in the ITF. This aligns with findings from Adegboyega et al. (2005) [16] and Zargaran et al. (2012) [14], noting diffuse Ki-67 expression throughout the malignant epithelium of TM in all OSCC cases.

Comparing MCM-2 and Ki-67 staining, the Mcm-2 mean nLI was higher than Ki-67 in all groups. Mcm-2 proteins are present during all cell cycle phases, detecting more proliferating cells than Ki-67, suggesting Mcm-2 is a more sensitive marker. Nowinska et al. (2010) suggested higher Mcm-2 expression compared to Ki-67 in the same tissue localization, as Mcm-2 may detect more proliferating cells

Table 6
Comparison of overall survival/disease free survival and treatment modalities in Group E (VSCC).

TREATMENT	SURVIVAL ESTIMATE	Std. Error	Lower CI	Upper CI	Log-Rank (Mantel-Cox)		
3 yr OS					Chi-sq	df	Sig.
(1) Surgery	42.385	1.824	38.809	45.960	7.033	1	0.008 S
(2) Surgery + Radiotherapy	36.000	0.000	36.000	36.000			
3 yr DFS					Chi-sq	df	Sig.
(1) Surgery	39.000	0.000	39.000	39.000	0.200	1	0.655 NS
(2) Surgery + Radiotherapy	39.706	0.679	38.374	41.037			

Table 7
Comparison of overall survival/disease free survival and treatment modalities in Group F (Conventional OSCC).

Treatment	Survival estimate	Std. Error	Lower CI	Upper CI	Log-Rank (Mantel-Cox)		
3 yr OS					Chi-Sq	df	Sig.
Surgery	42.667	1.959	38.827	46.507	3.900	1	0.048 S
Surgery + Radiotherapy	36.000	0.000	36.000	36.000			
3 yr DFS							
Surgery	39.000	0.000	39.000	39.000	0.229	1	0.632 NS
Surgery + Radiotherapy	40.465	0.901	38.699	42.231			

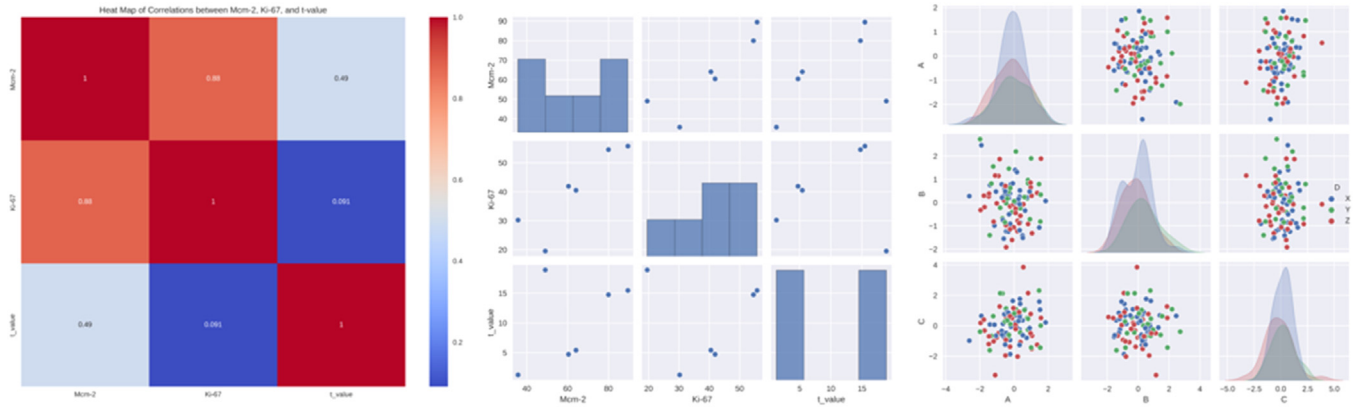

Fig. 5. Heat Map of Correlations, Pairwise Scatter Plots and Histograms, Pairwise Relationships with Categorical Variable Grouping of Mcm-2, Ki-67, and L-value.

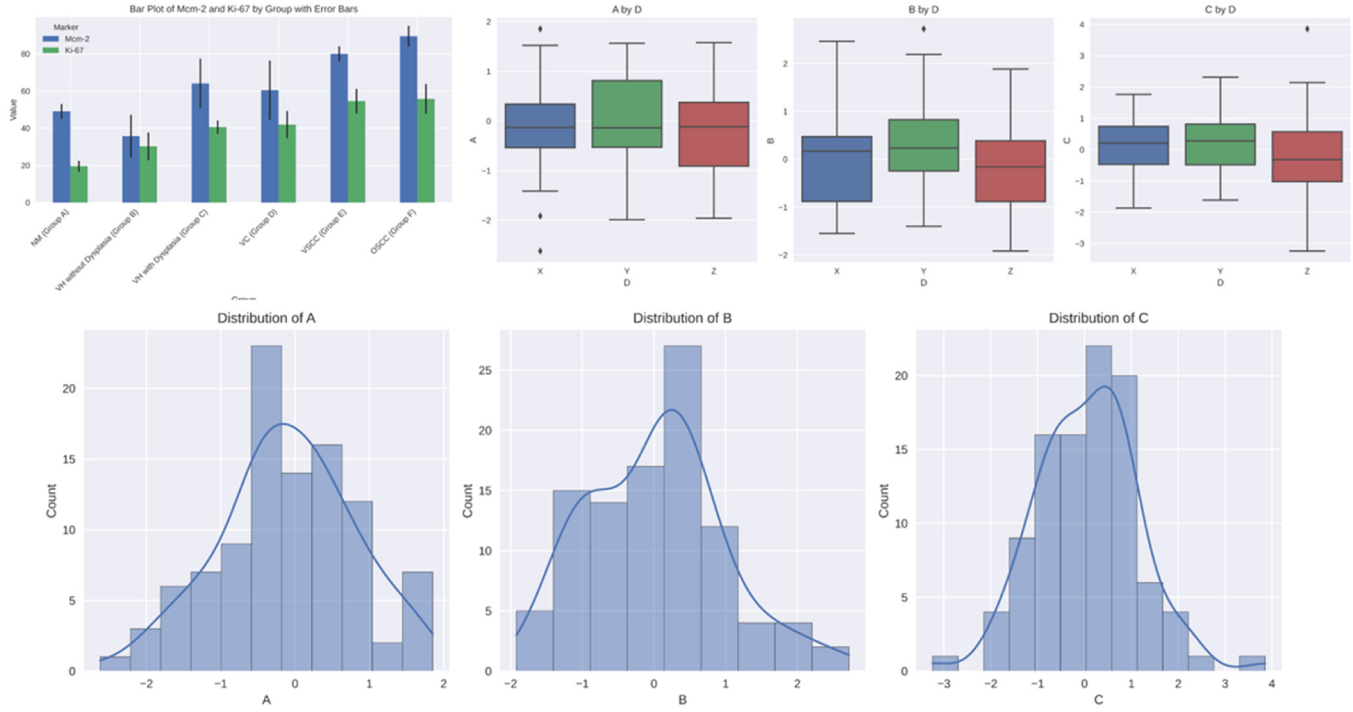

Fig. 6. Bar plot, box plot and distribution plots of the variables.

[22]. Similar observations were noted by Torres-Rendon et al. (2009) in oral epithelial dysplasia and OSCC [9].

Lesions within the buccal mucosa in Group E (VSCC) had lower OS than lesions on other oral sites, according to multivariate analysis with a Cox regression model (Table 2). Patients with habit associations (tobacco consumption, betel quid chewing, and combination of two or more habits) had reduced overall survival with statistical significance ($p < 0.05$). Cases with a DOI of 5–10 mm had reduced overall survival with statistical correlation ($p = 0.038$). Further studies could help prove independent parameters to predict prognosis and survival in VSCC.

In Group F (conventional OSCC), cases showing PVI and PNI had reduced overall survival ($p < 0.05$). Other clinicopathologic parameters did not yield a statistically significant correlation and could not show an independent role in predicting overall survival (Table 3). Studies by Oliviera et al. [23] and Watanabe et al. [24] showed age >60 years and anatomic site (tongue) were associated with reduced OS. PNI and PVI could be independent predictors for OS in OSCC patients, though more studies are needed.

The 3-year OS and DFS were compared to Mcm-2 and Ki-67 nuclear expression to assess their role in predicting survival in VSCC and conventional OSCC (Groups E and F). In VSCC (Group E), cases with higher Mcm-2 (>80.71 %) had reduced OS and DFS, whereas those below the cut-off (<80.71 %) had increased OS and DFS. Similarly, higher Ki-67 expression (>55.86 %) was associated with reduced 3-year OS and DFS compared to lower expression (<55.86 %). Although no statistical correlation was noted, further research could investigate the role of Mcm-2 and Ki-67 in predicting VSCC survival.

In conventional OSCC (Group F), cases with lower Mcm-2 expression (<89.9 %) had reduced 3-year OS and DFS (Table 5 & Fig. 3). Similar observations were noted for Ki-67 expression, with reduced 3-year OS and DFS in cases with higher expression (>89.9 %). Prediction of OS and DFS based on Mcm-2 and Ki-67 nuclear expression did not correlate with tumor behavior. A larger sample size and longer follow-up (5 or 10 years) could address this issue. Previous studies have not reported a role for proliferative markers (Ki-67, p53, PCNA) in OSCC survival (Koelbl et al., Watanabe et al., Bettendorf et al.) [24–26].

Comparing treatment modalities, Group E (VSCC) patients treated with only surgery had higher OS and DFS. Patients treated with surgery and radiotherapy had reduced OS and DFS (p = 0.008 & p = 0.048). Alkan et al. [27] and Wanjari et al. [28] reported wide surgical excision as the treatment of choice for non-invasive VC, with good prognosis and 80–90 % survival. VSCC cases require wide surgical excision and may necessitate aggressive surgical resection and radiotherapy. Combination treatments for VSCC need further research to clarify survival rates.

In Group F (OSCC), 3-year OS and DFS showed no significant correlation with treatment modality, aligning with findings by Watanabe et al. [24] and Oliviera et al. [23], indicating OSCC patients treated with surgery and radiotherapy had higher survival rates. They recommended combined therapy for OSCC patients with reduced survival rates. Although not statistically significant, the present study noted higher OS and DFS in OSCC patients treated with surgery alone (Table 7). To validate these results, more research with greater sample sizes and longer follow-up times is required.

In summary, the current study compared the overall or disease-free survival rates of individuals with VSCC and OSCC, as well as the lesions thought to be a part of a range of clinicopathologic disorders utilising the cell proliferation markers Mcm-2 and Ki-67. Higher Mcm-2 and Ki-67 expression was found in the study, which suggests a higher rate of proliferation in VSCC and OSCC. It is essential to determine clinicopathologic characteristics and putative markers of cell proliferation in order to forecast patient survival and prognosis. A greater number of samples and longer follow-up times are required for more research to validate these results and investigate the function of Mcm-2 and Ki-67 in VSCC and OSCC survival prediction.

5. Conclusion

The findings revealed higher expressions of Mcm-2 and Ki-67 in VSCC and OSCC, indicating increased cell proliferation. Clinicopathologic parameters such as anatomical site, habit associations, and depth of invasion significantly impacted overall survival in VSCC, while perineural invasion (PNI) and perivascular invasion (PVI) were critical in OSCC. Higher expressions of these markers were associated with reduced overall and disease-free survival, although statistical significance varied.

Treatment modalities also influenced survival rates, with VSCC patients undergoing surgical treatment showing better outcomes, while combined therapy with surgery and radiotherapy was more effective for OSCC patients. These results underscore the importance of differentiating between oral lesions and customizing treatment approaches accordingly. Further research with larger sample sizes and extended follow-up periods is necessary to confirm these findings and better understand the prognostic value of Mcm-2 and Ki-67 in VSCC and OSCC. This will enhance the prediction of patient outcomes and optimization of therapeutic strategies.

Statement of ethics

This research complied with the guidelines for human studies and includes evidence that the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The Subjects in this study does not require any informed consent as this is a retrospective study. This study protocol was approved by the Institu tional Review Board, SDM College of Dental Sciences and Hospital, Shri Dharmasthala Manjunatheshwara University (IRB. No.2015/P/OP/42).

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Declaration of competing interest

The authors declare that there is no conflict of interest.

CRediT authorship contribution statement

Niharika Abhay Sarathy: Conceptualization, Formal analysis, Methodology, Resources, Writing – original draft. **Kochli Channappa Niranjan:** Conceptualization, Formal analysis, Supervision, Validation, Writing – review & editing. **Devendra Alrani:** Formal analysis, Resources, Writing – review & editing. **Vani Niranjan:** Conceptualization, Formal analysis, Supervision, Validation, Writing – review & editing. **Nitya Krishnasamy:** Data curation, Formal analysis, Resources, Validation. **Vikram S. Amberkar:** Software, Supervision, Writing – review & editing.

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