RESEARCH

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Minichromosome maintenance-2 as a biological marker of oral epithelial dysplasia and squamous cell carcinoma

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ABSTRACT

Aims and Objectives: To evaluate the immunohistochemical expression of MCM2 in normal epithelium and in cases of oral epithelial dysplasia and squamous cell carcinoma and correlate MCM2 in different grades of dysplasia and different grades of oral squamous cell carcinoma. *Materials and Methods:* The study was carried to compare and evaluate the expression of MCM2 in OED and OSCC.

The study comprised of 15 cases of oral squamous cell carcinoma and 15 cases of oral dysplastic epithelium. The criteria used to define MCM2 positive cells were: brown staining of nuclei. 10 random fields were chosen and 100 cells were counted. *Results:* In different grades of dysplasia, the mean labeling index of MCM2 in different grades of dysplasia were calculated as 46.3 (SD=1.32), 50.325 (SD=4.47) and 60.44 (SD=6.58) respectively. These means were analyzed by using one way ANOVA test and p value of <0.001 was obtained which was statistically significant. In different grades of OSCC: the mean LIs of MCM2 in various grades of OSCC were calculated as 70.62 (SD=0.420), 79.02 (SD=2.456) and 83.65 (SD=0.494). These means were analyzed using one way ANOVA test and a p value of 0.000 was obtained which was statistically significant. *Conclusion:* This study indicated that MCM2 has a potential role to be used as a reliable proliferative marker in OED and OSCC. Its expression can be used not only to estimate the proliferative index, but also as a prognostic factor for the survival of patients with oral cancer.

Key words: Dysplasia**,** Immunohistochemistry, Minichromosome Maintenance-2, Squamous Cell Carcinoma, Tumor marker

1. INTRODUCTION

Cancer afflicts all the communities around the world. Approximately 10 million people are diagnosed with cancer and more than 6 million die of the disease every year. About 22.4 million persons were living with cancer in the year 2000. This represents an increase of around 19% in incidence and 18% in mortality since 1990 (Park K, 2007; Feller L, Lemmer J, 2012; Radhakrishnan R, Shrestha B, Bajracharya D, 2012; Arasteh & Seyedoshohadaei, 2019).

Cell proliferation plays an important role in several biological and pathological events. The proliferative potential can be assessed by Immunohistochemistry using monoclonal antibodies against specific cell cycle associated proteins. The cell cycles of normal and neoplastic cells are to known to be regulated by promoting factors including various cyclins and cyclin dependent kinases. The cell cycle is also regulated by minichromosome maintenance (MCM) proteins. These proteins were first discovered in yeast and constitute a family of at least six different nuclear proteins (MCM2-7) with striking sequence homology. (Babu GS et al, 2012; Kodani I, 2003; Karimi S, 2015)

The expression of MCM-2 in these may not only represent it as a proliferative marker but also as one of the prognostic marker. Hence this study further attempts to assess MCM-2 levels in oral epithelial dysplasia and squamous cell carcinoma.

2. MATERIALS AND METHODS

The present retrospective study was undertaken to compare and evaluate the expression of MCM2 in OED and OSCC. The study comprised of 15 cases of oral squamous cell carcinoma and 15 cases of oral dysplastic epithelium. It was carried out in formalin fixed paraffin embedded tissue sections of previously diagnosed cases of OSCC and OED which were retrieved from the archives of oral and maxillofacial pathology, Sri Sai College of Dental Surgery & PAHER university, Udaipur, Rajasthan. 15 normal oral mucosa tissue samples served as controls.

Inclusion criteria

1. Clinically and histopathologically diagnosed cases of OSCC.
2. Histopathologically diagnosed cases of OED.
3. Normal healthy subjects as controls.

Exclusion criteria

1. Patients with secondary OSCC metastasizing to oral cavity.
2. Patients undergoing chemotherapy and radiotherapy.

The antibodies and reagents used for immunohistochemical technique were obtained from Pathnsitu Biotechnologies Pvt. Ltd ready to use kit which consist of

1. Primary antibody – mouse antihuman Metallothionein
2. Secondary antibody – antimouse IgG
3. Peroxidase Block
4. Conjugate – Horse Radish Peroxidase
5. Chromogen substrate – Diaminobenzidine tetra hydrochloride (DAB)

Sectioning

4 micron thick sections were taken onto poly-L-lysine adhesive coated slide and incubated for 3 hour at 50-60 degrees centigrade in a slide warmer for proper adhesion of the section to the slide.

Evaluation of staining

Assessment of MCM2 positive cells was performed using double headed light microscope at 10x and 40x. MCM2 stains the mitotic figures of the nuclei. The criteria used to define MCM2 positive cells were: brown staining of nuclei. 10 random fields were chosen and 100 cells were counted.

Evaluation

Labelling index of MCM 2 marker for NOM, OED and OSCC were calculated using the formula

Labelling index (LI) = No. of immunopositive cell / Total no. of cells ×100

MCM2 expression level was evaluated using a semi quantitative scale (% of positive cells)

0 indicates "negative". i.e., no immune stained cells, +1 indicates "weak", i.e., < 25% immunostained cells,+2 indicates "moderate". i.e., 25%-50% immunostained cells and +3 indicates "strong" i.e., > 50% immunostained cells (Fig 1 -4).

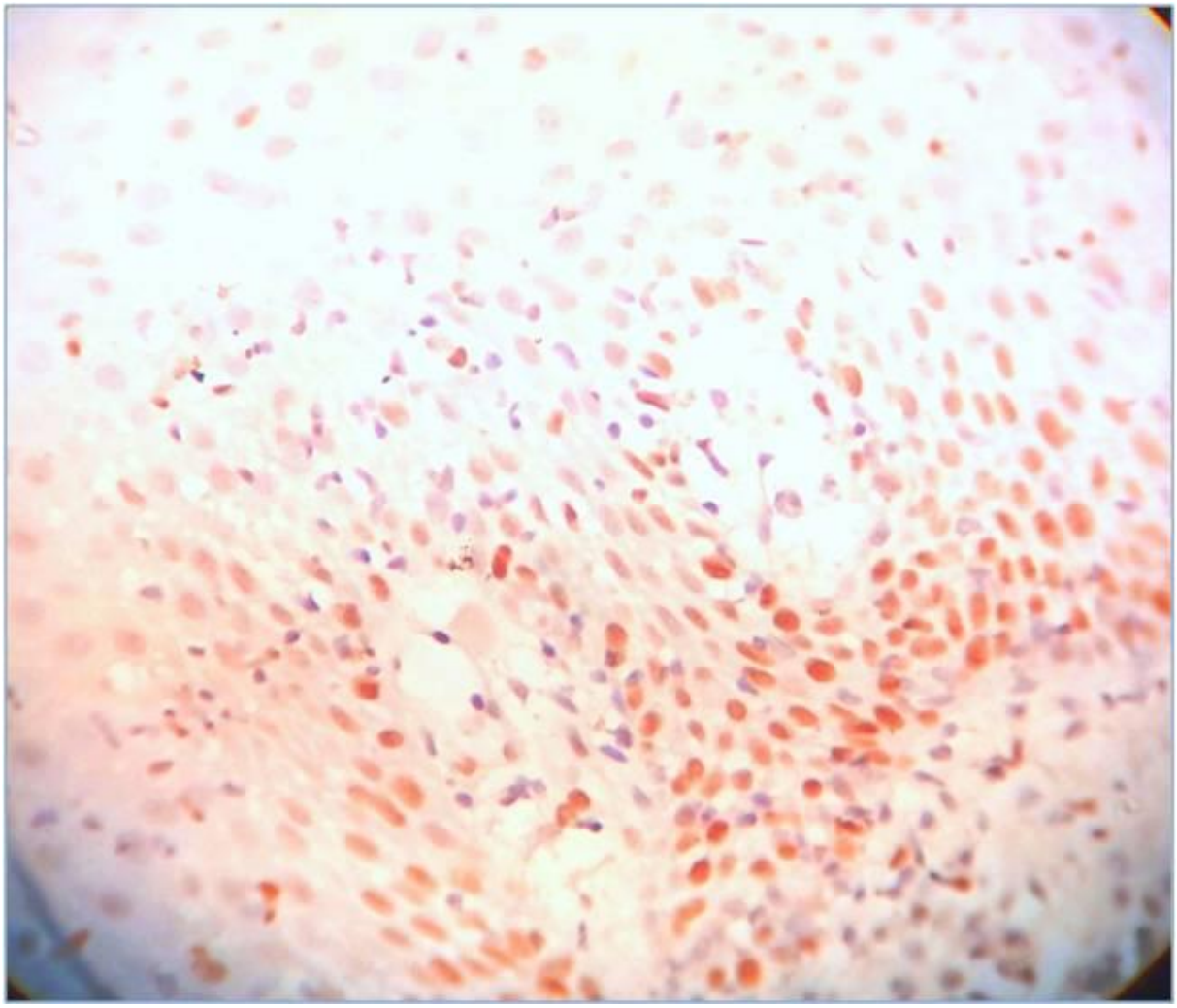


Figure 1 Mild Dysplasia

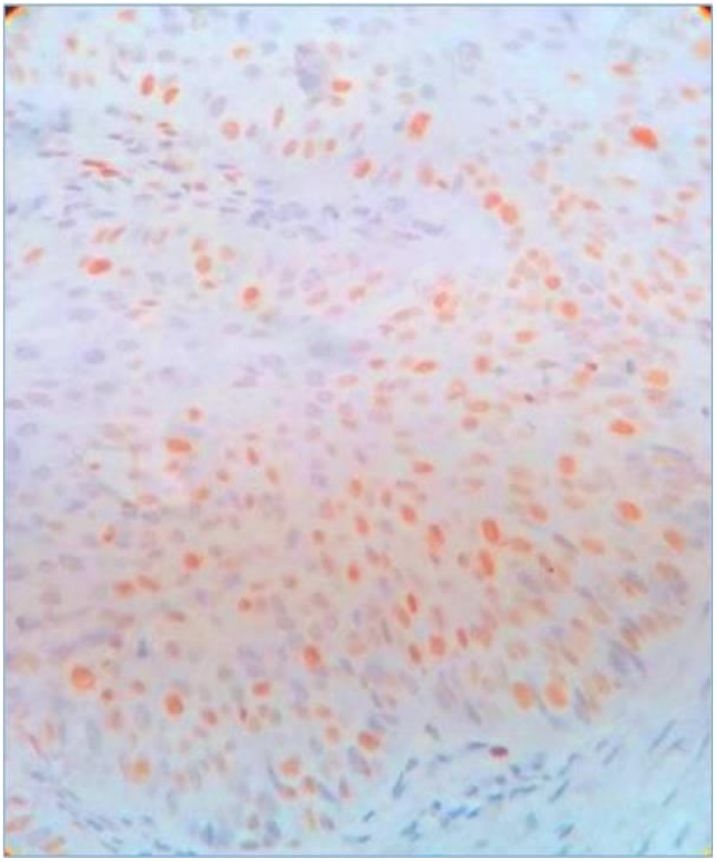


Figure 2 Moderate Dysplasia

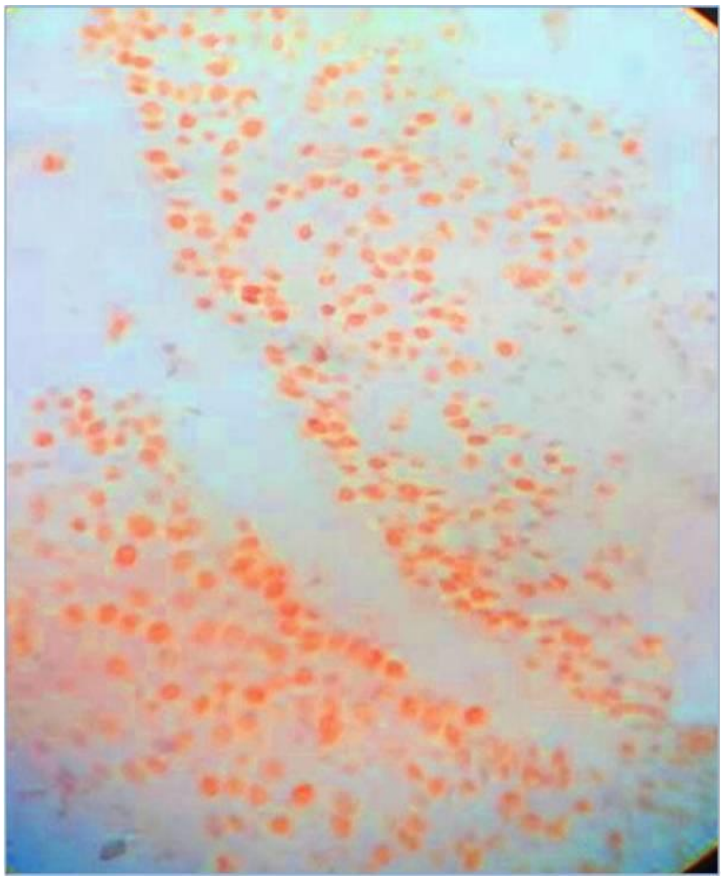


Figure 3 Severe Dysplasia

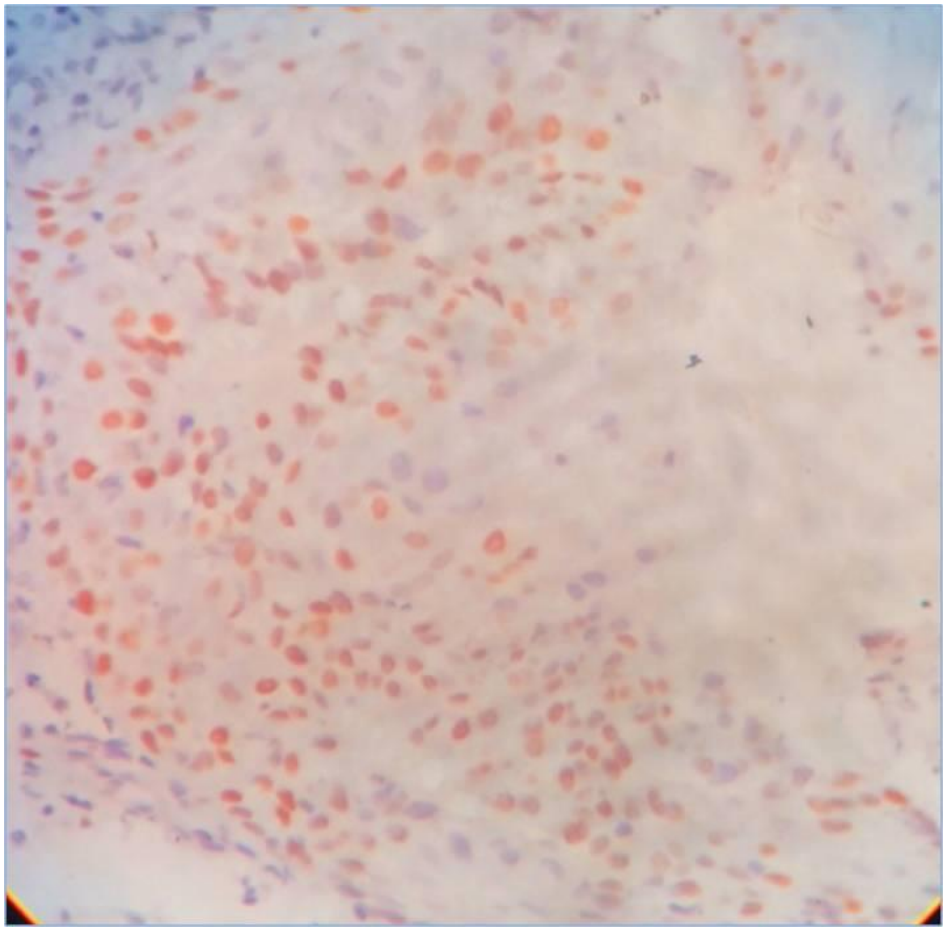


Figure 4 Moderate Grades SCC

Statistical analysis

All the calculations were performed using IBM SPSS (statistical package for the social sciences) statistical software package (SPSS 20.0, Chicago, IL, USA). The obtained data was subjected to ANOVA, chi-square test and one way ANOVA. A p value less than 0.05 was considered to be statistically significant. Inter observer variability was assessed using Pearson correlation and intra group correlation was done using ANOVA with Post-hoc tuckey's test.

3. RESULTS

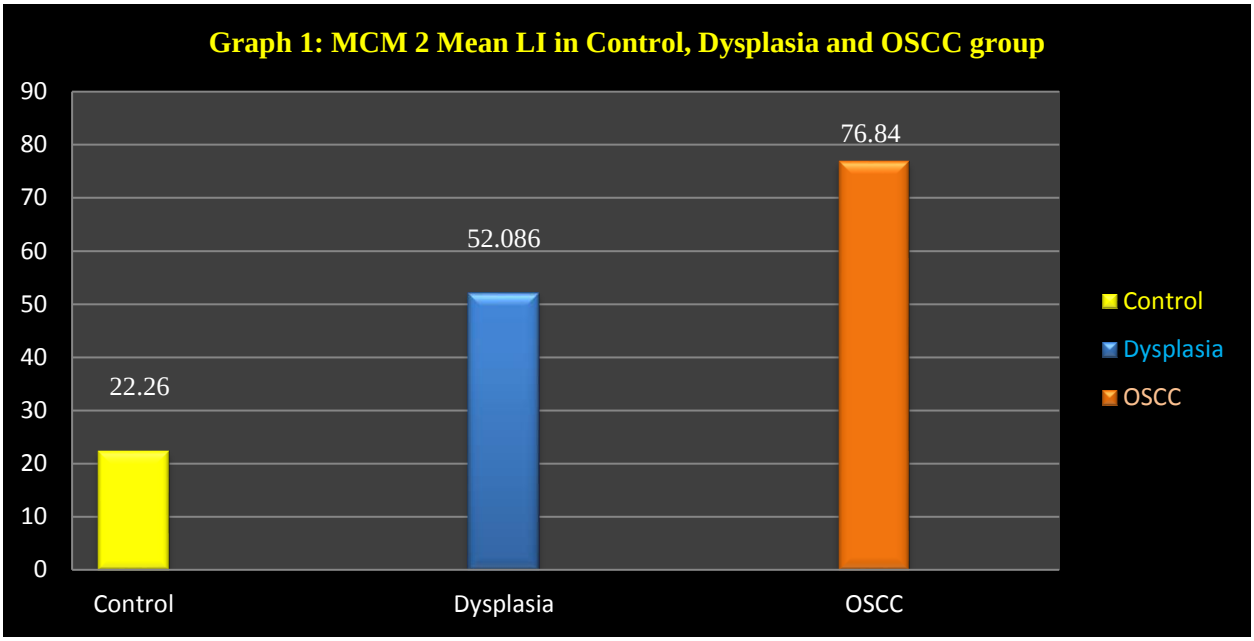
In our study, the inter observer reliability was assessed using Pearson correlation which was 0.860 and this was significant at 0.01 level, suggesting a significant strong correlation between observer 1 and observer 2. Hence the results obtained by observer 1 were taken into consideration for further statistical analysis.

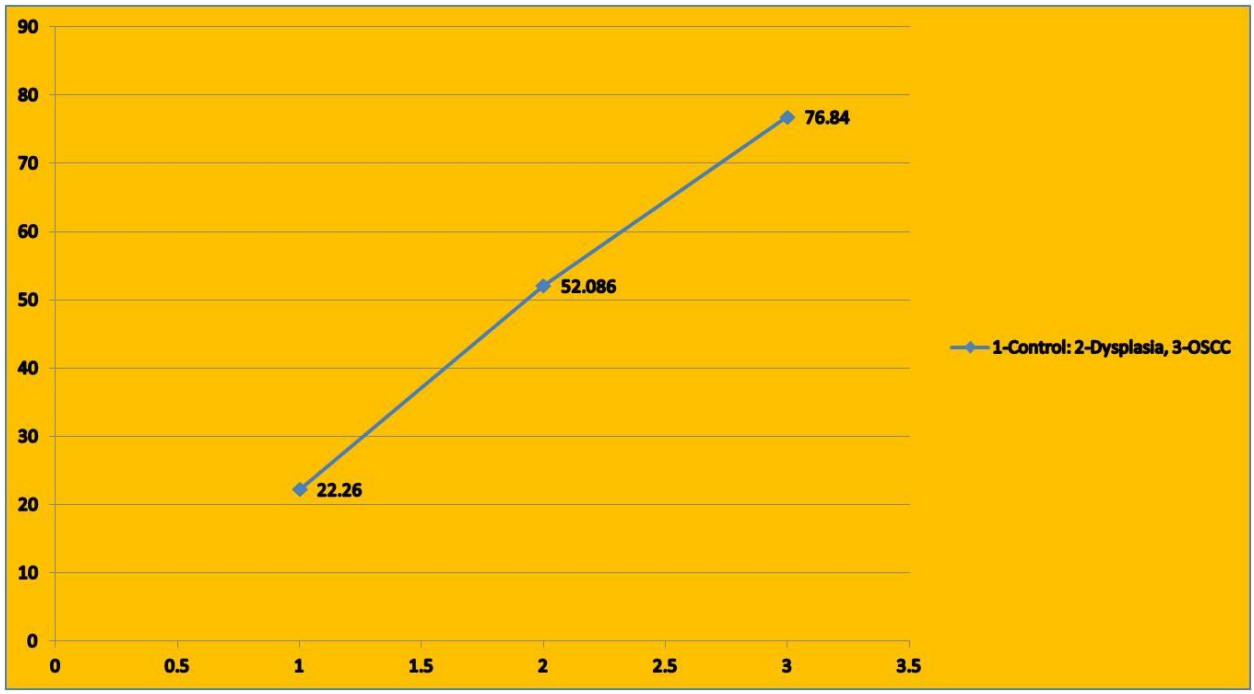
In the control group, a mean LI of 22.26 (SD=3.46) was obtained, while in the study group, a mean LI of 52.086 (SD=7.58) and 76.84 (SD= 5.12) was obtained for OED and OSCC respectively by using ANOVA test. P value of <0.001 was obtained which was statistically significant (Table 1 and Graph 1).

Table 1 MCM 2 Mitotic Index in Control, Dysplasia and OSCC

GROUP	TOTAL	MEAN	SD	P value
Control	15	22.26	3.46	0.000*
Dysplasia	15	52.086	7.58	
OSCC	15	76.84	5.12	

One way ANOVA Test. P value <0.05 (significant)





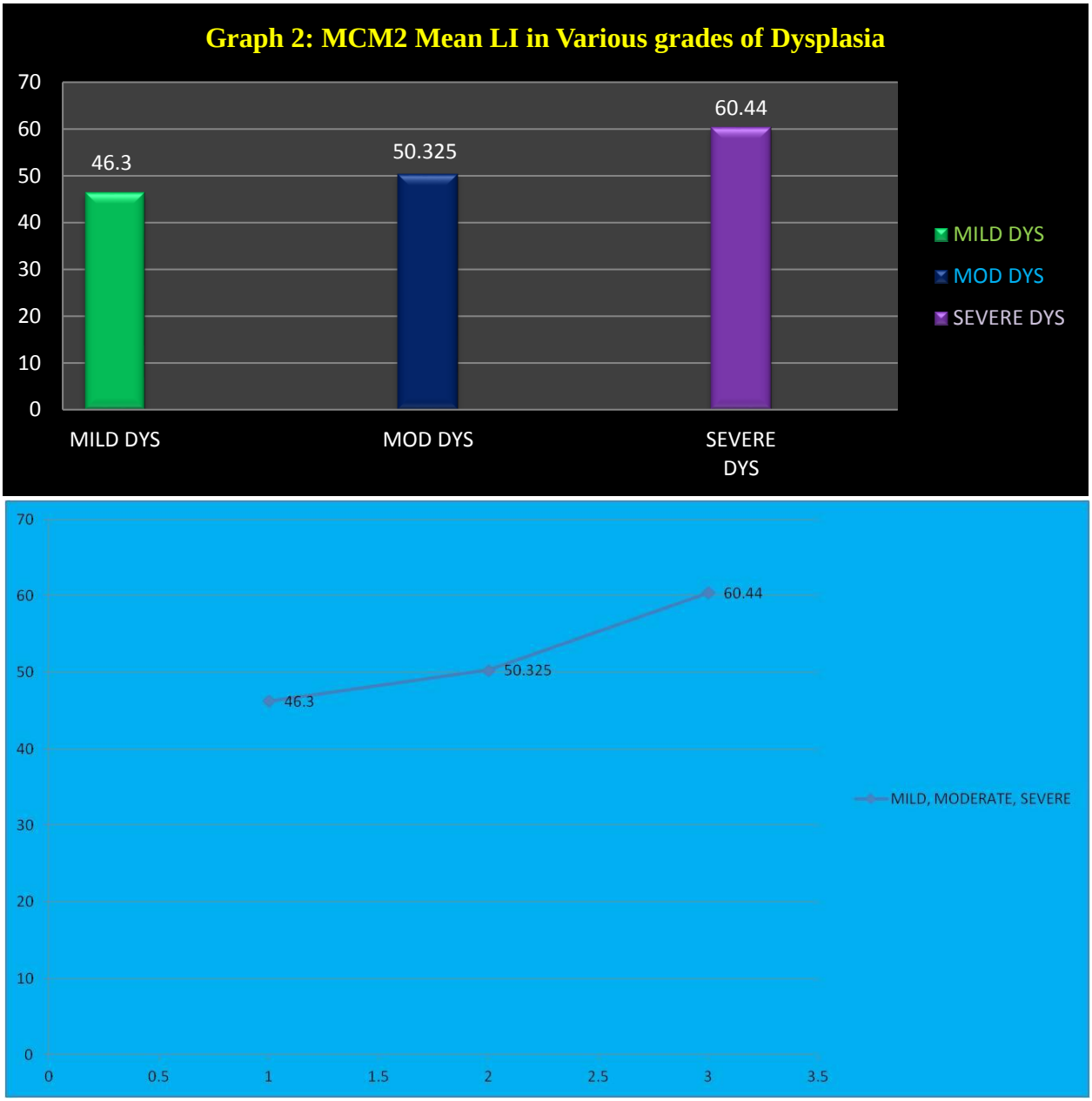
Graph 1 Mean Labeling index in three groups

In different grades of dysplasia, the mean LIs of MCM2 in different grades of dysplasia were calculated as 46.3 (SD=1.32), 50.325 (SD=4.47) and 60.44 (SD=6.58) respectively. These means were analyzed by using one way ANOVA test and p value of <0.001 was obtained which was statistically significant (Table 2 and Graph 2).

Table 2 Mcm2 Mitotic Index in Various Grades of Dysplasia

GROUP	Sample size	MEAN	SD	P value
MILD DYSPLASIA	6	46.3	1.32	0.001*
MODERATE DYSPLASIA	4	50.325	4.47	
SEVERE DYSPLASIA	5	60.44	6.58	

One way ANOVA test, P value <0.05 (significant)



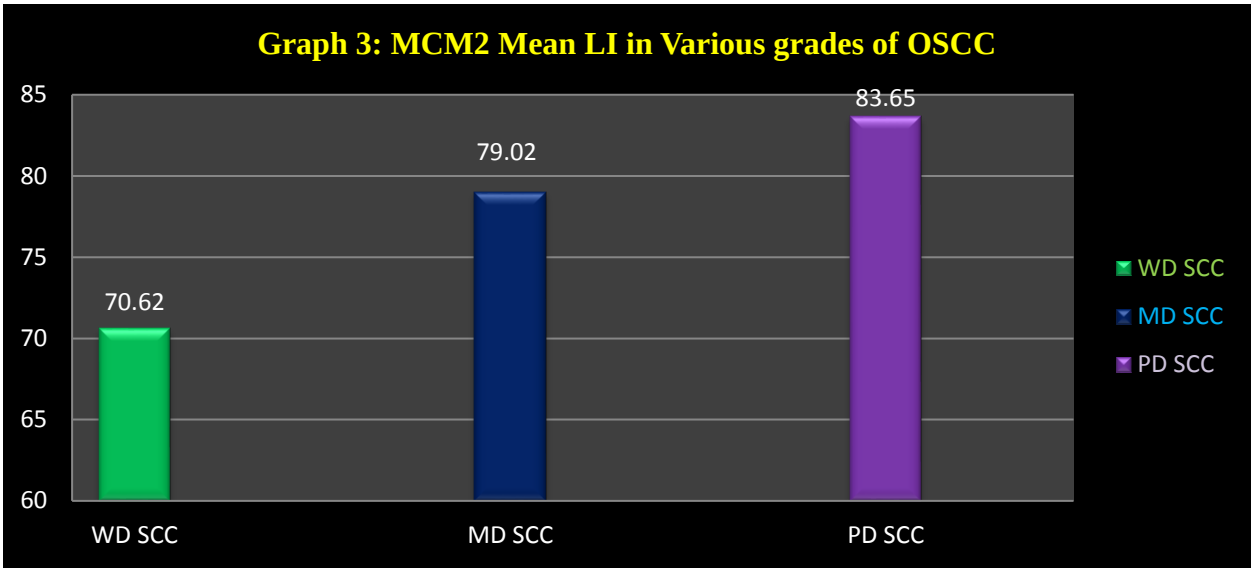
Graph 2 Mean labeling index in grades of dysplasia

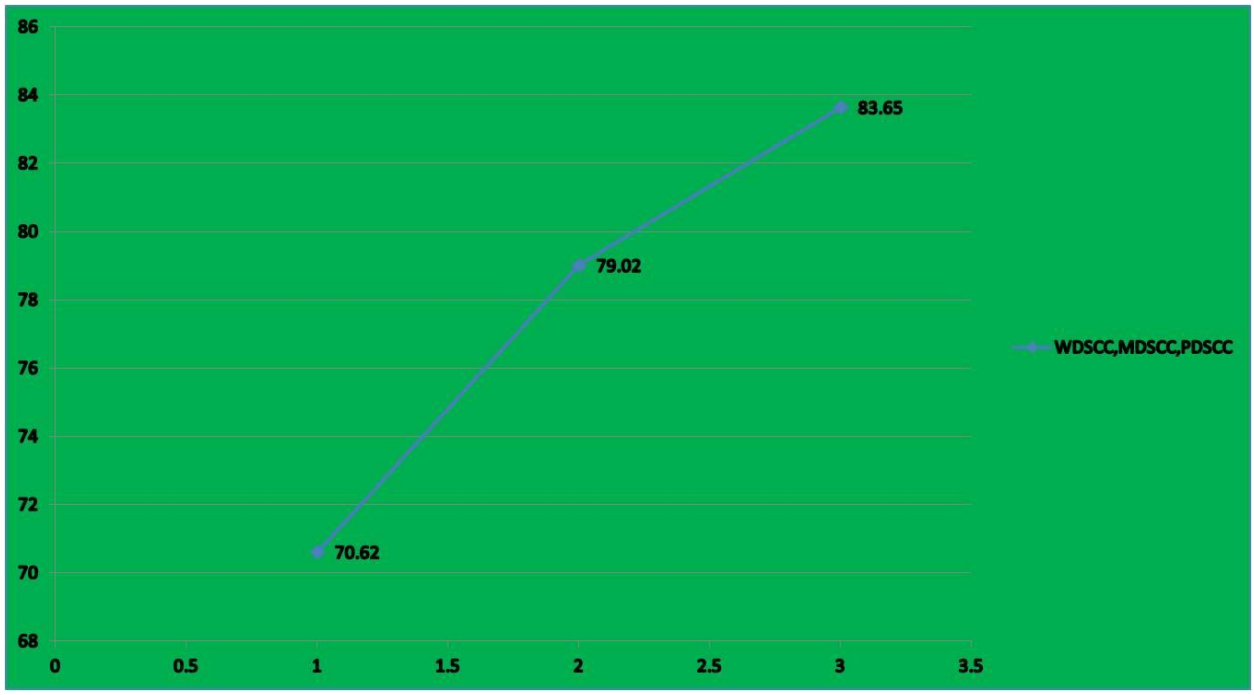
In different grades of OSCC: the mean LIs of MCM2 in various grades of OSCC were calculated as 70.62 (SD=0.420), 79.02 (SD=2.456) and 83.65 (SD=0.494). These means were analyzed using one way ANOVA test and a p value of 0.000 was obtained which was statistically significant (Table 3 and Graph 3).

Table 3 Mcm2 Mitotic Index in Various Grades of OSCC

GROUP	Sample size	MEAN	SD	P value
WDSCC	5	70.62	0.420	0.000*
MDSCC	8	79.02	2.456	
PDSCC	2	83.65	0.494	

One way ANOVA test, P value < 0.05 (significant)





Graph 3 Mean Labeling index in various grades of OSCC

Intra group comparison between different grades of OED and OSCC: It was done by using ANOVA with Post Hoc analysis using Tukey's test (Table 4).

Table 4 Intra Group Correlation of the Mean Mf's in different grades of Dysplasia and OSCC

GRADES	MEAN DIFFERENCE	P VALUE
MILD VS MOD DYS	4.025	0.378
MILD VS SEVERE DYS	14.14	0.001*, sig
MOD VS SEVEERE DYS	10.11	0.015*, sig
WDSCC VS MDSCC	8.405	0.000*, sig
WDSCC VS PDSCC	13.03	0.000*, sig
MDSCC VS PDSCC	4. 625	0.024*, sig

Post Hoc analysis using Tukey's test, p value < 0.05 (significant)

Assessment and analysis of MCM2 expression of MCM2 expression level in controls, OED and OSCC was assessed by using semi-quantitative method and the results were analyzed by chi- square test and p value obtained was 0.000 which was statistically significant (Table 5).

Table 5 MCM 2 Expression Level in Control, Dysplasia and OSCC

GROUP	SCORE					
	0	1	2	3	TOTAL	P value
CONTROLS	0	12	3	0	15	
DYSPLASIA	0	0	8	7	15	
OSCC	0	0	0	15	15	0.000*

Chi square test, P value < 0.05 (significant)

4. DISCUSSION

Neoplasms of diverse cellular origin arise in the oral cavity and among these OSCC arising from the mucosa constitutes to over 90. It is the sixth most common cancer worldwide. Most of the times, OSCC are preceded by a period during which the epithelium shows the evidence of epithelial dysplasia although this may not always be clinically apparent (Radhakrishnan R, Shrestha B, Bajracharya D, 2012; Kodani I et al., 2003; Razavi SM et al., 2015).

Tumor markers are substances that can be found in the body when cancer is present and can be detected in cells, tissue or body fluids, qualitatively or quantitatively by chemical, immunological or molecular biologic methods (Babu GS, 2012).

Recent studies have proposed that MCM proteins may be sensitive proliferation markers and may serve as novel biomarkers for prognostication and diagnosis of various premalignant and malignant lesions (Razavi SM, 2015). Therefore, the present study investigated the IHC expression of MCM2 in control group, OED and OSCC using LIs. When the results were evaluated, statistically significant relation (p value<0.05) was obtained between control group and study group.

In the current study, the immunochemical reactivity of MCM2 in the control specimens was expressed mainly in the basal and supra basal cells of the stratified squamous epithelium, with very few reactive cells in the middle third and a totally negative reaction in the superficial third. These results are in accordance with (Chatrath et al., 2003) and (Razavi et al., 2015) who also found similar expression. These findings hence indicate that cell division is confined to the basal and suprabasal cells, whereas the superficial cells have lost their proliferative ability.

In contradiction, (Rendon AT et al., 2010) found that MCM2 was located mainly in the suprabasal layer. The absence of MCM2 expression in a significant portion of basal cells was explained by assuming that these cells are in a temporary G0 state, as a part of self defence mechanism to maintain a controlled cell proliferation of the oral mucosa.

In the present study, it was observed that the IHC expression of MCM2 in OED was at a higher frequency in the basal and parabasal compartments and extended to mid prickle cell layer and in some cases to the surface layer. It was observed that the mean of LIs increased progressively, mild cases showed least expression (mean=46.3) followed by moderate (mean=50.325) and severe were showed the highest expression (mean=60.44). These differences were statistically significant (p value<0.001). These results are in accordance with (Chatrath et al., 2003) and (Razavi et al., 2015).

In cases of WDSCC and MDSCC in our study, positive immunoreaction was evident at the periphery of the epithelial cell nests and the invasive front, while core of the nests showed a negative reaction. These observations are in accordance with (Szelachowska et al., 2006) and (Scott IS et al., 2006), who also observed staining in peripheral cells of tumor islands. This highlights the active proliferative state of these tumor cells. However in poorly differentiated cases, the positive immunoreactions distributed all over the malignant epithelial cells, indicating the considerable proliferative behavior of OSCC of higher grades.

The findings indicated that the MCM2 expression was significantly higher in OSCC than OED and NOM. The mean LIs were calculated as 76.84, 52.086 and 22.26 respectively. These results were in accordance with (Razvi et al., 2015) and (Rendon AT et al., 2009) who also observed similar kind of expression pattern of MCM2.

MCM proteins are highly expressed in malignant human cancer cells and precancerous cells undergoing malignant transformation. They are not expressed in somatic cells that have been withdrawn from the cell cycle. Therefore, these proteins are ideal diagnostic markers for cancer and promising targets for anti cancer development.

5. CONCLUSION

In our present study, it was observed that overall the LIs of MCM2 was increased from NOM to OED to OSCC. This study indicated that MCM2 has a potential role to be used as a reliable proliferative marker in OED and OSCC. Its expression can be used not only to estimate the proliferative index, but also as a prognostic factor for the survival of patients with oral cancer. Considering its over expression in OSCC, there exists the possibility of applicability of MCM2 in molecular target therapy in patients with OSCC.

Finance resource & funding

None

Conflict of interest

Authors declare no conflict of interest

Ethical approval

All the authors hereby declare that all the experiments shave been examined & approved by the appropriate ethics committee (ETH/24/2018). Prior the participant's oral & written consent was obtained.

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