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MCM-2 expression differentiates potentially malignant verrucous lesions from oral carcinomas

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ABSTRACT

Background: Mcm-2 is a biomarker belonging to Mcm family of proteins which has rarely been used in oral potentially malignant and malignant lesions of the verrucous type. The objective of this study is to assess the expression of Mcm-2 in Normal Oral Mucosa (NM), Verrucous Hyperplasia (VH), Verrucous Carcinoma (VC) and Oral Squamous Cell Carcinoma (OSCC) and compare it with the clinicopathological characteristics.

Methodology: A total of 70 formalin fixed paraffin embedded tissue samples (10 cases of Normal Mucosa NM-Group A, 10 cases of Verrucous Hyperplasia- VH without Dysplasia- Group B, 10 cases of Verrucous Hyperplasia-VH with Dysplasia- Group C, 20 cases of Verrucous Carcinoma VC-Group D, 20 cases of Oral Squamous Cell Carcinoma OSCC- Group E) were subjected to immunohistochemistry with Mcm-2 antibody. Statistical analysis was carried out with various tests like ANOVA, Tukey HSD, Chi-Square and Shapiro-Wilk test by using the SPSS software.

Results: There was a significant difference in Mcm-2 expression with quantitative analysis among all the groups ($p < 0.05$). There was a significant progressive increase in nuclear Labelling Indices (nLI) from NM (49.08%), VC (60.45%), VH with Dysplasia (64.10%), and OSCC (89.22%).

Conclusion: The findings suggest that Mcm-2 may be a sensitive proliferation marker in oral potentially malignant and malignant lesions which may be useful for differentiating between VH with/ without dysplasia, VC and OSCC.

1. Introduction

Cell proliferation is a process which is vital to all living organisms. The control of this process is dysregulated in cancer, pre-cancer and hence these can be used as effective markers for early detection and prognosis of potentially malignant lesions and oral carcinomas. The commonly used cell proliferation markers include Ki-67, PCNA, Geminin, Cyclin D1, and Cyclin B1 for various oral lesions. But the literatures on these markers show that they have several limitations [1]. Hence there is an utmost need for a more sensitive proliferative marker to overcome these limitations. Mcm (Mini chromosome maintenance) group of proteins are such a group of cell proliferation markers which are a pre-requisite for DNA replication and cell cycle initiation and are expressed throughout the cell cycle. From this family of Mcm proteins, the preferred target for phosphorylation of Mcm hexamer is the Mcm-2 subunit which induces a conformational change in the Mcm complex and thus has an essential function in DNA replication [2]. Mcm-2 protein can be a favourable marker for early detection of altered abnormal cells in potentially malignant and malignant lesions having a tremendous potential for prognostication.

Oral verrucous lesions have been classified in the past and the most recent classification based on the nature of the lesion includes benign, potentially malignant and malignant verrucous lesions [3]. Verrucous hyperplasia, proliferative verrucous leukoplakia are classified as potentially malignant lesions, and verrucous carcinoma as a malignant lesion. Oral Verrucous lesions are clinically and histologically a diverse group of lesions as they present a difficulty in differentiating between these [4,5]. Although several conventional cell proliferation markers like Ki-67, PCNA, and Geminin have been used in the past to differentiate the potentially malignant lesions and malignant lesions, this is a first study reported using Mcm-2 as a marker in oral verrucous lesions (OVL).

Hence the present study aims to assess the expression of Mcm-2 in oral potentially malignant and malignant verrucous lesions and comparison with oral squamous cell carcinoma (OSCC).

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2. Methodology

A retrospective study was carried out on the routinely processed, paraffin-embedded tissue blocks obtained from the archives of the Department of Oral Pathology at SDM College of Dental Sciences, Dharwad. Clinical data were recorded retrospectively of the histologically diagnosed cases of Verrucous Hyperplasia (with and without dysplasia), Verrucous Carcinoma and Oral Squamous Cell Carcinoma. The Haematoxylin and Eosin sections were examined for histopathology and the cases were selected. The study was approved by the Institutional review board (No: 2015/P/OP/42) of SDM College of Dental Sciences and Hospital, Dharwad.

2.1. Immunohistochemical analysis

The paraffin embedded sections of 3 μm thickness from the blocks on silanized slides were collected and kept for overnight incubation. Sections were deparaffinised in xylene, rehydrated in alcohol. Antigen retrieval was carried out by heating in a pressure cooker using Tris-Edta buffer for 3 whistles. The sections were allowed to cool, after which incubation with 3% hydrogen peroxide was done to block the endogenous peroxidase activity following which incubation with primary antibody Mcm-2 (Biogenex, San Ramon, USA) was carried out for 1 h at room temperature. Further incubation with super-enhancer and secondary antibody (30 mins) was done followed by visualization by freshly prepared DAB (Diamino benzidine) chromogen for 10 min. The sections were not allowed to be dry until this stage and phosphate buffer saline (PBS) was used as a wash buffer after each step. The slides were then counter-stained with Harris Haematoxylin. The stained slides were examined by optical microscopy and the positive cell distribution in the different levels of epithelium was observed. Nuclear staining was considered as positive for Mcm-2.

2.2. Evaluation of Mcm-2 staining

The presence of brown coloured end product at the site of target antigen indicated positive-staining. Representative areas were selected in each case based on the highest number of positively stained nuclei. The manual cell count was performed using an eyepiece graticule × 10 oculars × 40 objective and a counting grid (Lawrence & Mayo) containing 100 blocks. The cases were scored by counting the positive cells per maximum of 500 tumor cells per case. The percentage nuclear labelling index (nLI) (No. of positive cells/total no. of tumor cells expressed as a percentage) was calculated per case. The intra and inter-observer reliability was assessed.

The stained slides were examined by an optical microscope, and the positive cell distribution in the different levels of the epithelium was also analysed. As shown in Fig. 6 the epithelium was divided into 3 levels: Level I (lower one-third of the epithelium i.e. Stratum Basale and Stratum Parabasal), Level II (lower two-third of the epithelium i.e. Stratum Basale, Stratum Parabasal and Stratum Spinosum) and Level III (extending to the upper 1/3rd of the epithelium i.e. Stratum Basale, Stratum Parabasal, Stratum Spinosum and Stratum Superficiale or Stratum Corneum). The positively stained nuclei were counted as described earlier in all the 3 levels and nuclear labelling indices were calculated in Groups B, C and D [14]. (See Figs. 1–4.)

2.3. Statistical analysis

Chi-square test was performed to compare the association of patient's clinicopathological parameters and nuclear labelling indices (nLI) of the Mcm-2 proteins whereas, Kolmogorov–Smirnov, Shapiro–Wilk test were done to evaluate the distribution of the tests. Tukey post-hoc test was also done to compare the nuclear labelling indices between the above mentioned groups A to E. The nLI of Mcm-2 was compared with the patient's age, gender, site, tumor size and betel-quid, tobacco habits with chi-square test. SPSS software version 20.0 was used for statistical analysis. A value of $p < 0.05$ was considered to indicate a statistically significant result.

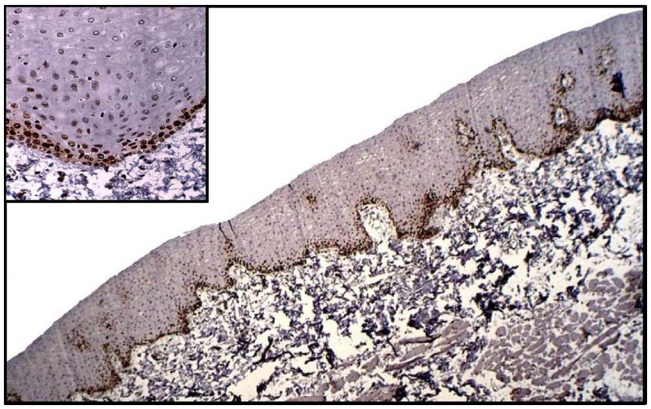

Fig. 1. (NM) Stratified squamous epithelium showing nuclear staining of Mcm-2 in basal and parabasal cell layer. (IHC-Monoclonal Anti-body, Mcm-2; Original Magnification 10×, inset 40×).

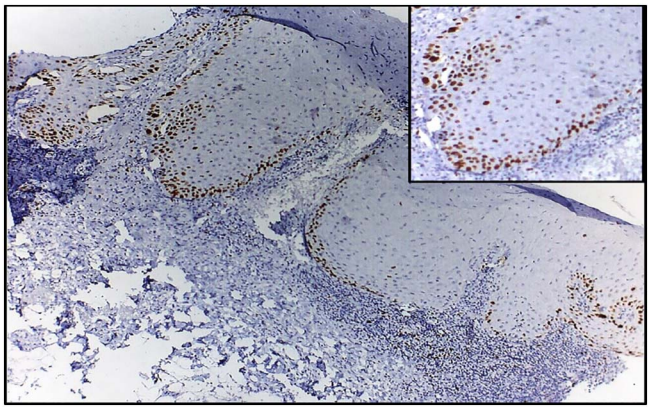

Fig. 2. (VH without Dysplasia) Hyperkeratinized stratified squamous epithelium showing prominent uniform nuclear staining of Mcm-2 in the basal, parabasal cell layers with randomly stained cells in the suprabasal cell layers. (IHC-Monoclonal Anti-body, Mcm-2; Original Magnification 10×, inset 40×).

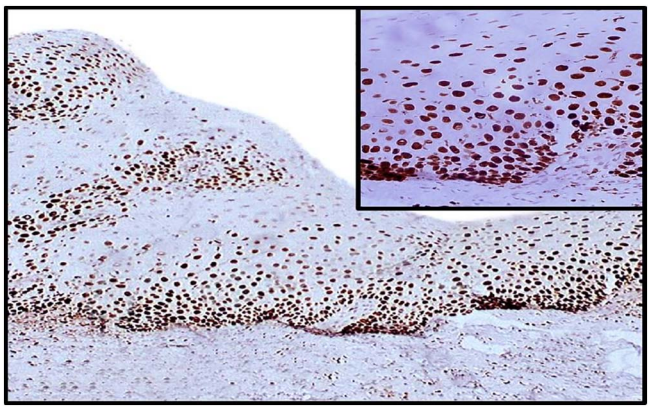

Fig. 3. (VH with Dysplasia) Hyperkeratinized dysplastic stratified squamous epithelium showing prominent nuclear staining of Mcm-2 in the basal to superficial cell layers involving > 2/3rd of the epithelium. (IHC-Monoclonal Anti-body, Mcm-2; Original Magnification 10×, inset 40×).

3. Results

This study comprised of 70 patients whose clinicopathological