



## Evaluation of Minichromosome Maintenance-3 (MCM3) in Erosive Oral lichen planus (An immunohistochemical study)



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### Abstract:

The present study evaluated the immunoexpression of MCM3 in oral erosive lichen planus. Ten patients having erosive oral lichen planus (EOLP) and ten normal mucosa from patients undergoing minor oral surgery were subjected to immunohistochemical analysis to evaluate MCM3 expression. All EOLP showed positivity for MCM3. The amount of positive cells increased as the grade of dysplasia increased.

**Keywords:** erosive oral lichen planus, MCM3, oral cancer.

### Introduction

Oral cancer is considered one of the most common cancer types worldwide <sup>(1)</sup>. Typically, oral squamous cell carcinoma (OSCC) is preceded by oral potentially malignant disorders (OPMD), which are defined as "morphologically altered tissue in which oral cancer is more likely to occur compared with its normal mucosae" <sup>(2)</sup>. Oral leukoplakia, erosive oral lichen planus, oral submucous fibrosis, and oral erythroplakia are the most common diseases of the oral mucosa that have a great malignant transformation rate <sup>(3)</sup>.

Oral lichen planus is a potentially malignant disorders which may appear in several clinical forms such as reticular, papular, plaque-like, atrophic, bullous and erosive type. Erosive and atrophic subtypes have increased malignant transformation rate compared to others <sup>(3)</sup>.

Oral potentially malignant disorders are usually histologically classified according to the presence or absence of oral epithelial dysplasia <sup>(4)</sup>. Early diagnosis of these lesions is very important and can be life-saving, as they may progress to severe dysplasia, carcinoma in situ and/or squamous cell carcinoma in their late stages <sup>(3)</sup>. The identification of reliable biomarkers for detecting malignant transformation poses a unique role for the development of standardized screening and improved follow up in patients with oral precancerous lesions <sup>(5)</sup>.

Proliferation markers have been broadly used to detect various human malignancies. MCM3 is one of the members of minichromosome maintenance protein family with a vital role in DNA initiation and replication <sup>(6)</sup>. It appears during proliferation of malignant, premalignant and normal cells, and but absent in cells that are in G0 phase of cell cycle <sup>(7)</sup>. The basic role of MCM proteins in replication of DNA and their decreasing role in quiescent cells might give them the function of a proliferative marker in screening of cancer <sup>(8)</sup>.

### Aim of the work:

The aim of this study was to assess the immunohistochemical expression of MCM3 in erosive oral lichen planus.

### Patients and methods:

The selected subjects were divided into two groups. Group 1 included 10 cases of erosive oral lichen planus. Exclusion Criteria include smokers and alcohol abusers, and lichenoid

reaction or any therapy for lichen planus in the previous three months. Group 2 included ten normal mucosa from patients undergoing minor oral surgery.

Clinical assessment including age, sex, site and duration was performed to all patients. Biopsy was taken from all cases for confirmation of diagnosis. Then assessment of dysplastic changes was performed. Finally, immunohistochemical analysis was performed.

### Histopathological Assessment:

The incisional biopsies from the lesions were stained by haematoxylin and eosin and examined by transmitted light. The degree of epithelial dysplasia was evaluated. It was divided into mild, moderate, severe or carcinoma in situ. The criteria for dysplasia that was established by WHO include architectural and cytologic changes in the epithelium. Architectural changes include irregular stratification of the epithelium, loss of polarity of basal cells, drop-shaped rete pegs, increased mitotic figures, abnormal and atypical mitosis, and keratin pearls within rete pegs. Cellular changes are: pleomorphism of the nucleus, pleomorphism of the cells, increased nuclear-cytoplasmic ratio, and dyskeratosis <sup>(9)</sup>.

In mild dysplasia, dysplastic changes are restricted to the lower third of the epithelium. Changes are found up to two-thirds of of the epithelium in moderate dysplasia while in severe dysplasia, changes occupy more than two-thirds of the epithelial thickness, but less than the whole epithelial thickness. dysplasia occupies the whole thickness of the epithelium in carcinoma insitu but the basement membrane remains intact <sup>(10, 11)</sup>.

### Immunohistochemical Assessment:

For immunohistological analysis, the sections were cut to three to four millimeters thickness then mounted on slides coated with poly-L-lysine. Deparaffinization of sections using xylene was performed. Rehydration in alcohol was done followed by retrieval of antigen. The activity of endogenous peroxidase was stopped with 3% hydrogen peroxidase/methanol. The sections were incubated with a mouse monoclonal antibody against MCM3 as primary antibody then, the slides were rinsed gently with PBS and the secondary antibody. Incubation with 3,3- diaminobenzidine

tetrahydrochloride (DAP) was done for ten minutes as a substrate chromogen solution (peroxidase antiperoxidase method) (12).

Cervical high-grade squamous intraepithelial lesions were used as the positive control disease. Negative control was established by replacement of the primary antibody with PBS (12).

Immunohistochemical results was evaluated under a light microscope and scored as follows (12):

- ❖ 0: no detectable staining (<5%),
- ❖ 1: (5% and <25%),
- ❖ 2: (25% and <75%)
- ❖ 3: (>75%).

### Results:

Group 1 (erosive oral lichen planus group) included 10 patients, 7males and 3 females with mean age (54.20±8.88). Group 2 (normal oral mucosa group) included 10 patients, 7males and 3 females with mean age (48.40±10.94). There were no statistical significant differences in age and sex among studied groups (p=0.21). All cases of oral erosive lichen planus in this study occurred in buccal mucosa.

Among the studied cases of erosive oral lichen planus, mild dysplasia was seen in 6 cases of erosive oral lichen planus. Moderate dysplasia three cases of erosive oral lichen planus. Severe dysplasia was seen in only one case of erosive oral lichen planus.

MCM3 expression was seen to be restricted to the nucleus in all samples. There is immunoeexpression variation among groups. Expression of MCM3 was significantly higher in erosive oral lichen planus compared to normal oral mucosa (P<0.001).

In normal oral mucosa, eight samples were positive for MCM3 staining and only two samples were negative (< 5% of stained cells). In cases of normal epithelium, MCM3 expression was restricted to the nuclei of basal cell layer.

All samples of erosive oral lichen planus were positive for MCM3 staining. In oral erosive lichen planus, MCM3 expression differs according to the degree of dysplasia. The amount of stained cells increased as the grade of dysplasia increased, and the immunostaining was seen in any cell layers of the epithelium. In mild epithelial dysplasia, MCM3 expression is restricted to the lower third of the epithelium while in severe epithelial dysplasia, positively stained nuclei were present throughout the epithelium.

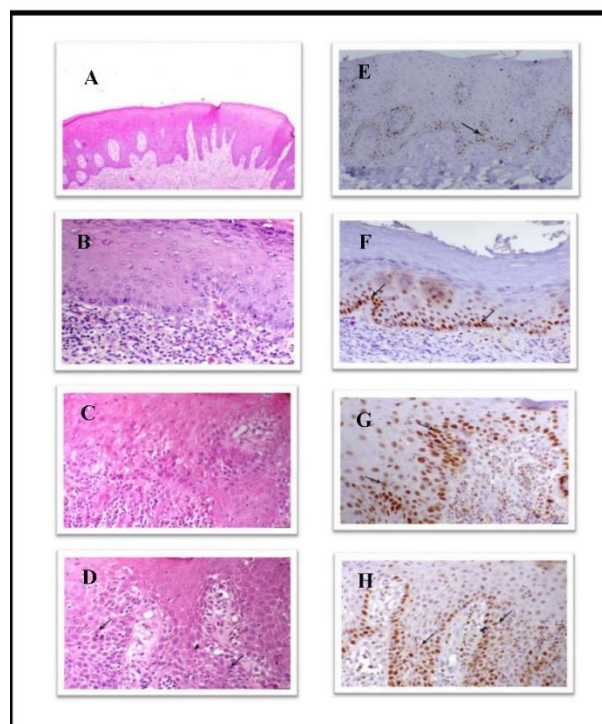


Figure (1): Histopathology and immunoeexpression of MCM3 in normal oral mucosa and oral lichen planus (A, E) Normal oral mucosa. (B, F) Oral lichen planus with mild dysplasia. (C, G) Oral lichen planus with moderate dysplasia. (D, H) Oral lichen planus with severe dysplasia.

### Discussion:

Oral squamous cell carcinoma is the sixth most frequently diagnosed malignancy with a high incidence of morbidity and mortality worldwide (13). It is generally believed to be preceded by potentially malignant disorders such as oral leukoplakia and erosive oral lichen planus (14).

Knowing the mechanism of malignant conversion is very important as it aids the clinician in evaluation of the malignant transformation risk of these premalignant lesions, and so preventive strategies can be performed. Therefore, in this study we evaluated immunohistochemical expression MCM3 in relation to clinicopathological parameters of studied cases of erosive oral lichen planus in order to have proper information about clinical significance of this marker.

The mean age of erosive oral lichen planus cases was (54.20±8.88). This accords to Simarpreet et al. (15) who stated that the mean age of oral lichen planus was 56.2 years and range was 35-67 years.

Regarding sex, in erosive oral lichen planus patients, males were more affected than females. This accords to Munde et al. (16) who observed that the men outnumbered the women (ratio M: F = 1.61:1) but in contradiction with Xue et al. (17) who reported that the male to female ratio was 1:1.91.

In this study, MCM3 expression was increased from normal epithelium to severe dysplasia. In normal epithelium, the staining was limited to the nuclei of basal cells and a few cells in the immediate suprabasal layers. In dysplastic epithelium, there was a direct correlation between grades of

dysplasia and MCM3 expression. With increase in grades of the lesion, MCM3 expression and staining score have increased as well. These results came in agreement with [Valverde Ludmila et al.](#) <sup>(18)</sup>, and [Lameira et al.](#) <sup>(19)</sup> who consider that MCM3 is superior to Ki-67 in evaluating dysplastic oral lesions.

These results were also in accordance with [Torres-Rendon et al.](#) <sup>(20)</sup> and [Kodani et al.](#) <sup>(21)</sup> who reported a positive correlation between dysplastic grades and MCM3 expression. Also these results accords to [Revanzi et al.](#) who reported that MCM3 expression increased from normal to dysplastic epithelium and from dysplastic epithelium to SCC.

[Ibarra et al.](#) showed that a complete complex of MCM is necessary to protect the integrity of genome against natural replication stress during S phase <sup>(22)</sup>. So, the higher expression of MCM3 in higher grades of dysplasia in this study may propose the same defensive mechanism against genomic injury and before malignant transformation <sup>(20)</sup>.

To this end, studies have indeed reported that increased MCM3 expression is associated with a worse prognosis in salivary gland tumors <sup>(23)</sup>, thyroid carcinoma <sup>(24)</sup>, and melanoma <sup>(25)</sup>. In OSCC and premalignant oral lesions, MCM3 is regarded as a prognostic and diagnostic marker <sup>(12)</sup> that some consider to be superior to Ki-67 <sup>(19)</sup>.

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