

## Original Article

# Comparative analysis of cell proliferation ratio in plaque and erosive oral lichen planus: An immunohistochemical study

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## ABSTRACT

**Background:** Proliferating cell nuclear antigen (PCNA) is a nuclear protein synthesized in the late G1 and S-phase of the cell cycle. Detection of this protein represents a useful marker of the proliferation status of lesions. This study has been carried out to evaluate the cell proliferation rate in oral lichen planus (OLP) and comparison between plaque and erosive lichen planus, which indicates the potential for malignant transformation.

**Materials and Methods:** This study was comprised of 64 cases of histologically proven lichen planus, out of which 32 cases of plaque and erosive each was taken. Two sections were taken from each, one for H and E staining to verify histological diagnosis according to Eisenberg criteria, other sections were stained according to super sensitive polymer horse radish peroxidase method for identifying immunohistochemical expression of PCNA. Data were statistically analyzed by Tukey high-range statistical domain test. Statistically significant *P* value was considered <0.05.

**Results:** In two types of lichen planus, erosive type (66.86%) showed higher expression of PCNA followed by plaque (17.07%). Overall, *P* value was <0.001, which was statistically significant. It indicates that proliferation activity is more in erosive lichen planus followed by plaque type, which ultimately results in increased rate of malignant transformation.

**Conclusion:** PCNA is a good nuclear protein marker to evaluate the proliferation status of OLP. Out of the two types of lichen planus, erosive type possesses more proliferative ratio and chances of malignant change is more in this type. It emphasizes the importance of long-term follow-up with erosive type when compared with plaque type.

**Key Words:** Immunohistochemistry, oral lichen planus, proliferating cell nuclear antigen

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## INTRODUCTION

Oral lichen planus (OLP) is a relatively common, chronic inflammatory mucocutaneous disease. This mucocutaneous disease was first described by Wilson in 1869. OLP affects 1-2% of the world's population<sup>[1,2]</sup> and 1.5% in Indian population.<sup>[3]</sup> It is

a disease of middle age, but occasionally children are also affected.<sup>[1]</sup> Oral lesions are characterized by raised multiform white lesions accompanied by areas of erosion and pigmentation.<sup>[3]</sup>

Since the first case of gingival cancer was reported in a patient with OLP in 1910, it has become the focus of much controversy. Many studies have attempted to assess the malignant transformation potential of OLP. These studies have suggested that a lesion originally diagnosed as OLP has 6.51% possibility of undergoing malignant transformation in time. Based on these studies, the World Health Organization has classified OLP as a potentially malignant disease.<sup>[3,4]</sup> Some authors have however argued that such transformation has not been sufficiently documented to justify this

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classification. According to these authors, more precise criteria are needed to diagnose OLP, especially from a histopathological standpoint. For these authors, most of the cases of malignant transformation could not be considered as such, as there were already alterations that suggested malignancy upon the initial diagnosis of lichen planus.<sup>[2,5]</sup>

Age and gender of patient are not related to the increase of the risk of malignant transformation of OLP. The true malignant transformation of OLP can be evaluated by analyzing the expression of proteins related to cell proliferation and apoptosis, as alterations in these proteins are essential for carcinogenesis.<sup>[6-10]</sup> Proliferating cell nuclear antigen (PCNA) is considered as a useful protein marker to assess the proliferation status of lesions. Higher the cell proliferation rate, the higher risk of malignant transformation. In context, OLP with increased PCNA can have a higher malignant transformation risk. Among different forms of OLP, erosive type is found to have malignant potential when compared with plaque. Therefore, the aim of this study was to evaluate the immunohistochemical expression of PCNA in OLP in order to explain the controversy regarding the potential for malignant transformation of OLP and emphasize the importance of long-term follow-up of patients with this disease.

## MATERIALS AND METHODS

### Study design

A total of 64 cases of previously diagnosed OLP, 32 cases of each plaque and erosive were collected. Cases of lichenoid dysplasia and lichenoid reaction were excluded from the study. Histological sections were prepared from paraffin embedded blocks. One section was stained with H and E to verify the histological diagnosis according to Eisenberg criteria. Other sections were stained according to super sensitive polymer horse radish peroxidase method for identifying immunohistochemical expression of PCNA. Diagnosed lymphoma cases were taken as a positive control for PCNA expression and for negative control the primary antibody was omitted during the immunohistochemical staining.

Eisenberg histopathological criteria for the diagnosis of OLP, included as essential and non-essential findings.<sup>[11]</sup>

Essential findings are the presence of

- Liquefied baseline layer.

- Intense lymphocyte infiltration in layers underlying the epithelium with effacement of the baseline layer.
- Normal epithelial cell maturation.

Other findings (non-essential)

- Interpapillary crests in a “sawtooth” shape.
- Hyperparakeratosis.
- Civatte bodies.
- Separation of the epithelium of the lamina propria.

### Exclusion criteria

- Cells with large and/or hyperchromatic nuclei.
- Presence of dyskeratosis.
- Increased number of mitoses or atypical mitoses.
- Projection of epithelial “drop-like” cones.
- Absence of liquefied baseline layer.
- Loss of epithelial stratification.
- Heterogeneous inflammatory infiltrate.
- Extension of infiltrate to deeper layers.
- Perivascular infiltrate.

Thousand cells were counted in all epithelial layers of both types of lichen planus (Basal, intermediate and superficial layers). It was considered positive when more than 5% of cells stained and negative when less than 5% of cell stained. In a slide, 3-4 fields were selected and counted [Figures 1 and 2]. Data was statistically analyzed by Tukey high-range statistical domain test. Statistically significant *P* value was considered <0.05.

## RESULTS

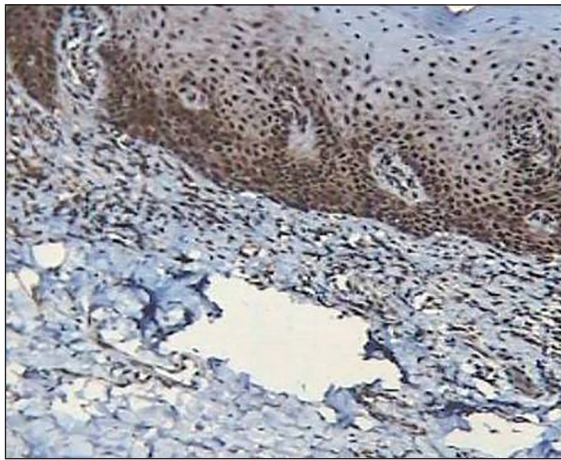
Of the 64 cases of lichen planus, 100% were positive for PCNA. Out of two variants, erosive type showed a higher expression of PCNA (66.86%) compared with plaque type (17.08%) [Table 1]. In erosive type, minimum expression was 55% and maximum expression was 80.30%. In plaque variety, minimum expression of PCNA was 5.6% and maximum expression was 25.6%. Overall *P* value was <0.001, which is statistically significant [Figures 3 and 4].

In erosive type, PCNA expression is more in the intermediate layer followed by basal layer and

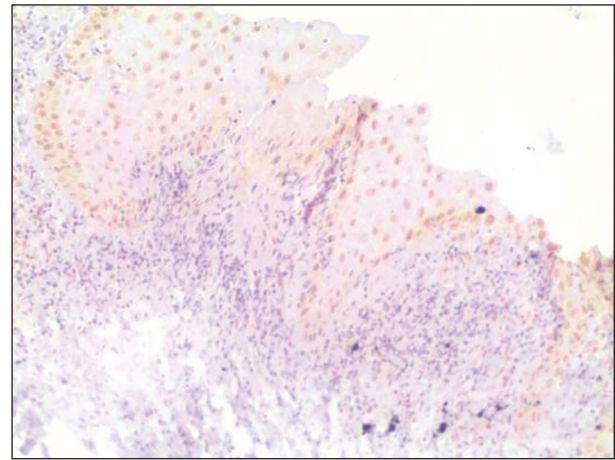
**Table 1: Expression of PCNA in percentage**

Type of OLP	N	Mean	Standard deviation	Minimum expression (%)	Maximum expression (%)
Plaque	32	17.0750	6.30793	5.60	25.60
Erosive	32	66.8625	9.65898	55.00	80.30

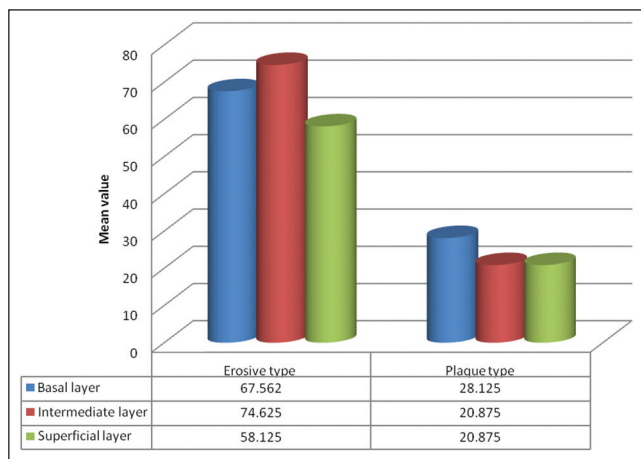
*F*: 150.143, *P* < 0.001 (statistically significant *P* value is <0.05); OLP: Oral lichen planus; PCNA: Proliferating cell nuclear antigen



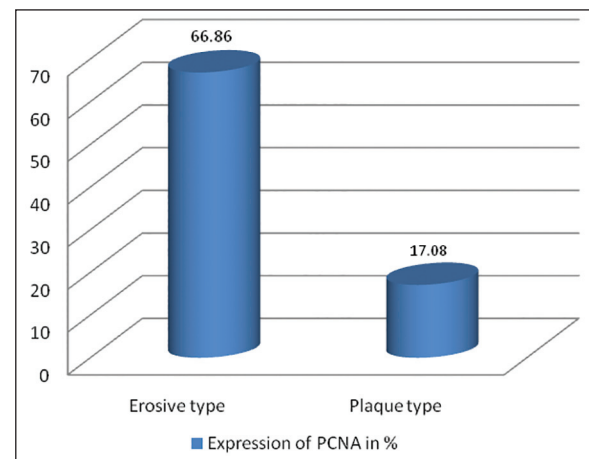
**Figure 1:** Photomicrograph showing proliferating cell nuclear antigen expression in basal, intermediate and superficial layer of oral lichen planus



**Figure 2:** Photomicrograph showing proliferating cell nuclear antigen expression in basal, intermediate and superficial layer of type of oral lichen planus



**Figure 3:** Expression of proliferating cell nuclear antigen in percentage



**Figure 4:** Comparison of expression of proliferating cell nuclear antigen in percentage in all three layers

superficial layer. In plaque type, PCNA expression is more in the basal layer followed by superficial and intermediate with equal expression.

## DISCUSSION

OLP is the most common dermatologic disease affecting the oral mucosa with unknown etiology,<sup>[1]</sup> with the prevalence rates ranging from 1% to 2% in the general population.<sup>[5]</sup> Predominantly, it affects middle-aged patients and 60-65% of patients are female.<sup>[6]</sup> OLP may be found in any location of the mouth, but favored sites are the bilateral buccal mucosa, whereas the tongue, gingiva and palatal lesions are more uncommon.<sup>[7]</sup> Clinically, OLP has specific and clearly identifiable features and presents with reticular, erosive, papular, plaque-like, bullous and atrophic forms.<sup>[8]</sup> Patients with OLP frequently

present one or more extraoral lesions. About 25% of women with this disease also present concomitant vulvovaginal mucosal involvement and about 15% of all patients with this disease also have skin lesions.<sup>[9]</sup> Skin lesions have been classified as polygonal, pruritic and purple papules.<sup>[10]</sup> Histopathologically, plaque type shows thick layer of hyperkeratosis, inflammatory cell infiltration will be mild and band like inflammatory cell infiltration will be present in the lamina propria. Erosive type shows ulcerated and atrophic epithelium, and a broad eosinophilic band is present below the covering epithelium.<sup>[12]</sup>

Many studies have shown that OLP can undergo malignant transformation, controversy still exists as to whether OLP should be considered a premalignant condition.<sup>[13-15]</sup> Alterations in the expression of proteins related to cell proliferation and apoptosis

are a strong indicator of the malignant transformation potential of certain lesion.<sup>[15]</sup> PCNA is an enzyme related to the proliferative state of the cell because of its close association to components of the cell cycle. However, the PCNA expression may be associated with DNA repair processes or be stimulated by such growth factors as cytokines. Thus, because of its long half-life, PCNA may be detected in cells that have left the cell cycle.<sup>[16,17]</sup>

Chiang *et al.* concluded that the gradual increase of PCNA expression with the morphologic transformation of normal epithelial cells into dysplastic epithelial cells suggests that there is increased proliferative activity in oral premalignant lesions with disease progression.<sup>[18]</sup> In our study, expression of PCNA is more in erosive type of lichen planus. We also found that PCNA expression is more in all three layers of epithelium, when compared to plaque type, this suggests that there is increased proliferative activity in erosive type of lichen planus.

Mignogna *et al.* has suggested that currently there is sufficient evidence demonstrating that chronic inflammation, which is the case of OLP, generates a cytokine-based microenvironment that affects cell survival, growth, proliferation and differentiation; this may consequently contribute to cancer initiation, promotion and progression.<sup>[19]</sup>

Study conducted by da Silva Fonseca *et al.*, suggest that the keratinocyte proliferation index is higher in lichen planus than in keratosis and normal mucosa.<sup>[20]</sup> This was in accordance with the present study.

Xue *et al.* concluded that about 0.65% of the 674 patients developed epidermoid carcinoma in sites with a previous diagnosis of erosive lichen planus, suggesting that increase in the risk of malignant transformation in erosive form. In our study, expression of PCNA is more in erosive type of lichen planus. This might result in increased risk of malignant transformation.<sup>[21]</sup>

Mitamura *et al.* found that expression of PCNA was higher in reticular and plaque type than atropic type of OLP. In contrast, our study showed that the expression of PCNA to be more in erosive type followed by plaque type. The discrepancy of contrasting findings has been explained by Mitamura *et al.* to be due to different immunohistochemical methods or different measurement methods employed in PCNA expression studies.<sup>[12]</sup>

Lee *et al.* investigated the expression of the p53 protein and the PCNA in OLP and its relation with the clinical behavior of the disease and the habits of patients. They found no significant correlation between the expression of both proteins and any clinical feature of OLP.<sup>[22]</sup>

## CONCLUSION

PCNA is a good marker to indicate the proliferative status of disease. Its expression may also be associated with DNA repair process or can be stimulated by growth factors. Out of the two types of lichen planus, erosive type possess more proliferative ratio, hence chances of malignant change is more in this type. This study emphasizes the importance of long-term follow-up with erosive type when compared with plaque type due to this increase malignant transformation potential.

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