ORIGINAL RESEARCH

Expression of Bax in Oral Lichen Planus – An Immunohistochemical Study

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ABSTRACT: **Background:** Oral lichen planus (OLP) affect a minor percentage of the general population, but the morbity caused by this disease is a cause of concern. Apoptosis is known to occur during the course of OLP. **Aims and Objectives:** To find the expression of Bax using immunohistochemistry and To find its role in the pathogenesis of oral lichen planus. **Methodology:** The present study was done using Immunohistochemical procedure on the 21 samples procured from the archival material. The pro-apoptotic marker Baxwas demonstrated and analysed using peroxidase – antiperoxidaseimmunostainingmethod. ***Results*:** The results were analysed using the positivity of the immunostaining of the samples. Further the positivity was graded in terms of their staining intensity, distribution and location of stain uptake. The results were subjected to statistical analysis and analysed using a non-parametric analysis, Mann Whitney test and was found to be statistically significant with p value of 0.000. **Conclusion:** The results obtained in this study suggest that the process of apoptosis occurs in OLP. Hence inhibition of apoptosis in the patients could reduce the severity of the lesions and thus representing new specific targets for treatment of Lichen Planus.

Key words: Oral Lichen Planus, Apoptosis, Bax, Immunohistochemistry

Oral lichen planus (OLP) occur in approximately 2% of the general population while 10-20% of patients demonstrate oral as well as cutaneous lesions. It is a T-cell-mediated chronic inflammatory oral mucosal disease of unknown etiology. OLP lesions contain few B-cells or plasma cells and minimal deposits of immunoglobulin or complement.[(1)] The characteristics of OLP are hyperkeratosis, basal layer vacuolization with apoptotic keratinocytes, and a mononuclear cell infiltrate at the epithelium-connective tissue interface.[(2-4)] Apoptosis is a programmed cell death that can be caused by the cell itself, the surrounding tissue, or a cell that is part of the immune system. It is also effective in preventing cancer.[(5,6)] Previous studies on apoptosis in OLP are based on histology and detection of markers for apoptosis. The triggers for apoptosis in OLP are still unknown, but it has been speculated that the attack by inflammatory T

cells might be crucial. The lichen planus antigen as of now is still unknown, and considered to be a self-peptide, thus classifying lichen planus as an autoimmune disease. It has been identified that heat-shock protein (HSP) expression is up-regulated by OLP lesional keratinocytes. The role of heat shock proteins in the pathogenesis of Lichen Planus has been substantiated, thus supporting the concept of Lichen Planus as an autoimmune disease, where these protein molecules may act as a foreign antigen.[7,8] Some studies have reported that apoptosis can thus be triggered and occurs not only in basal and parabasal epithelial cells, but also in the subepithelial inflammatory cell infiltrate.[9-13] It has also been suggested that low frequency of apoptosis measured as Bax expression in epithelial cells of OLP may favor malignant transformation [14] The main aim of the present study was to assess the expression pattern of Bax in OLP lesions.

AIMS AND OBJECTIVES:

To evaluate the expression of Bax in OLP lesions

To find its role in the pathogenesis of OLP.

MATERIAL AND METHODOLOGY:

The archival samples (21 in number) which were histopathologically diagnosed as OLP were included for the study. The procured paraffin -embedded samples were sectioned to the thickness of 3-4µm and mounted on coated (charged) slides. Then the sections were deparaffinised and routine immunohistochemical staining procedure using peroxidase – antiperoxidase method was followed.

Immunohistochemistry: Immunohistochemistry was used to detect the expression of Bax in the collected samples. Anti- Bax protein (6 ml) used was polyclonal – ready to use, obtained from rabbit polyclonal antibody which required no antigen retrieval. The procedure was standardized using both the positive and negative controls. The positive control used was breast carcinoma tissue for Bax (Cytoplasmic) and the negative control was the normal gingiva. The primary antibody anti -bax was incubated for 30 minutes, diaminobenzidine (DAB) was used as the chromogen and mallory'shematoxylin for counterstaining the nuclei. The sections were washed and dehydrated with grades of alcohol, mounted and viewed under the microscope for the evaluation of the results.

Evaluation of Immunostaining

Each section was examined under light microscopefor the expression of Bax individually, based on the following criteria: intensity of staining (mild, moderate and severe/intense); distribution pattern of the stain (localized or generalized/diffuse); location (layers of epithelium stained). (Fig. 1,2,3). Out of 21 samples, 17 were immunopositive for Bax with 4 samples showing negative results.

RESULTS AND OBSERVATIONS:

The results were then subjected to statistical analysis. The results obtained using the staining features like intensity of staining, distribution pattern, location were tabulated and statistically analyzed using non-parametric test called Mann – Whitney test. The results were statistically significant in relation to the staining intensity, distribution and staining location with p value of 0.000. (Table 1& Fig 4).

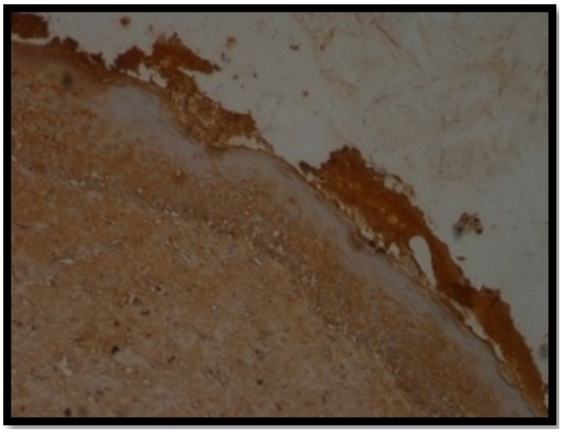

Fig 1: Photomicrograph of mild uptake of the stain in 10X magnification.

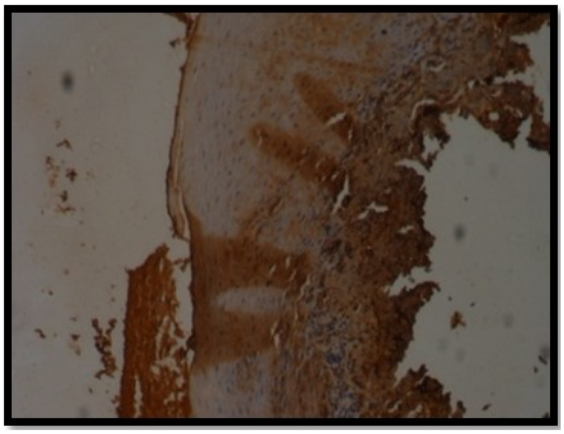

Fig 2: Photomicrograph of moderate uptake of the stain in 10X magnification.

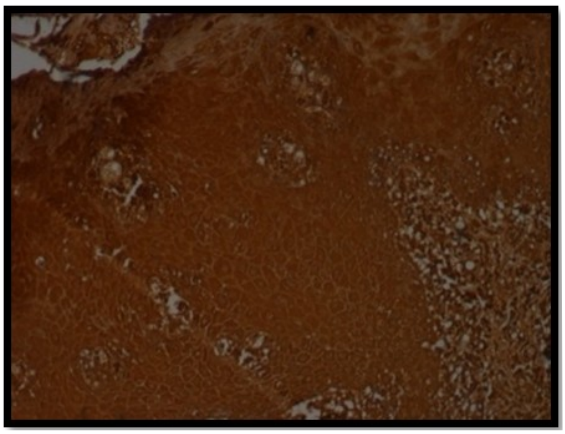

Fig 3: Photomicrograph of severe/intense uptake of the stain in 10X magnification.

DISCUSSION:

Oral mucosa is affected by many mucocutaneous lesions and can sometimes be an initial manifestation of the disease process. Oral Lichen Planus is one such lesions associated with cutaneous manifestations and may be an initial manifestation of the disease process, even before cutaneous manifestations appear.[15] It is a well-known fact that apoptosis plays a key role in the pathogenesis of this disease and numerous studies have supported the role of apoptotic markers through the disease process.[16] The etiology of Lichen planus is unclear, however auto-immune as an etiological genesis is considered, hence immunohistochemical analysis was employed as a tool to assess the same.[17]

Apoptosis occurs in a variety of settings. It is often difficult to estimate the extent, because of the rapidity with which the apoptotic cells and fragments are taken up by adjacent parenchymal and/or mesenchymal phagocytes. Understanding of the mechanisms of cell death is rapidly increasing with a sequence of biochemical events that culminates in cell death. Bax, a pro-

Value	Intensity of staining in Oral Lichen Planus	Distribution pattern in Oral Lichen Planus	Location of staining in Oral Lichen Planus
Mann Whitney U value	64.500	76.000	62.000
P value	.000	.000	.000

Table 1: Mann Whitney U test

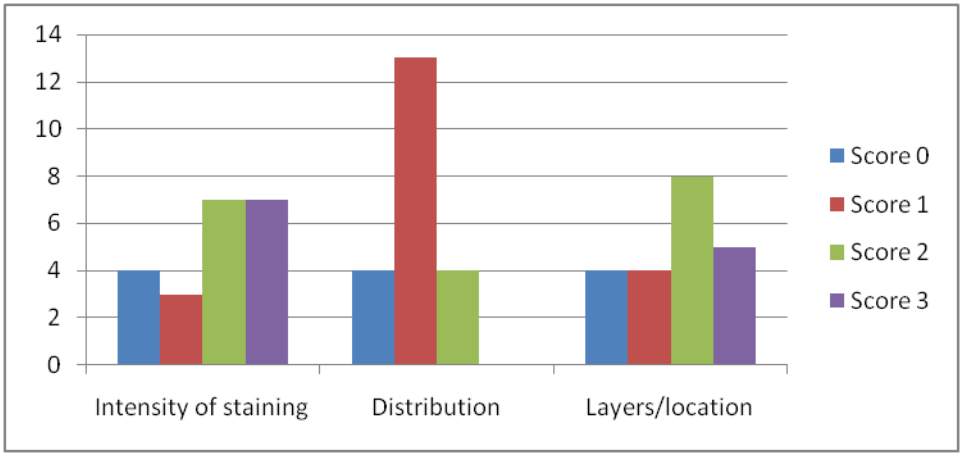

Fig 1: Data analysis of immunopositivity is depicted based on 3 features related to staining: Intensity, Distribution and Location.

apoptotic protein is a member of Bcl-2 family, promotes the release of cytochrome c which normally resides exclusively in the intermembrane space of the mitochondria and thus plays a role in the activation of caspase-9. Activated caspase-9, being an initiator caspase, activates an effector caspase, such as caspase-3, through proteolytic cleavage.[18]

Fernando A.C. G de sousa et al in their study in 2008 compared the expression of proliferative markers with the apoptotic markers and found the expression of apoptotic marker Bax to be low. They deducted that alterations in the expression of proteins related to the cell proliferation and apoptosis were strong indicator of the malignant transformation potential of these lesions. With the increased proliferative marker expression it could be concluded that the malignant transformation was high and increased expression of apoptotic marker could suggest low malignant transformation potential.[19] Hence, we can infer that in our study, high expression of Bax in Oral Lichen Planus indicates its low malignant transformation potential.

Xu'e Chen et al in 2008 in their study investigated the immunohistochemical expression of Bax in skin lesions of Lichen Planus and reported that 93.1% of their study sample to be positive for bax. Thus they inferred that Bax overexpression in Lichen Planus reflects its important role during the period of apoptosis in lichen planus. It is suggested that the high expression of Bax can block the activity of Bcl-2.[20] ShimaNafarzadeh et al in 2013 in their study analysed Oral Lichen Planus samples for Bax expression and found to be positive[21] and their study results correlated with our study results also.

Apoptosis being also a part of normal physiologic process, mild immunopositivity may also be considered as a part of the physiological process.[22] However, in epithelium, layers involved in this process and the distribution of the stain uptake may largely determine the physiologic or pathologic status of the phenomenon. Apoptosis may be a normal phenomenon in the superficial layers of the epithelium. But the immunopositivity in the basal and parabasal or spinous layers can be a definitive indication of apoptosis in the pathological condition. The variations in the staining intensity, distribution and the location may thus be an indication of the complexity of interactions through which the apoptotic proteins initiate the process and may be indicative of the present stage

of the disease process. Thus it may be helpful to monitor the progression of the disease process.

Therefore in this study the expression of apoptotic marker, Bax gains importance in the background of its potential for malignant transformation. Bax activity suppresses the anti-apoptotic BCl- 2 activity. A. Basu and S. Halder have elaborated in their review on the relationship between Bcl 2, Bax and p53 and stated that Bax promotes cell death by competing with Bcl2.[23] This clearly states that in our study samples the strong positivity of Bax is suggestive of its lower potential for malignant transformation. This adds value to the conflicting reports regarding the malignant potential of Oral Lichen Planus where some studies have claimed it to be considered as a potentially malignant disorder.

CONCLUSION:

The result of this study well confirms the role of apoptosis in OLP which imparts its role in a therapeutic intervention. Skin lesions are often transient in nature, whereas lesions of oral LP may demonstrate a chronic and protracted clinical course. Although it is still subject to some controversy, LP may have premalignant potential. Therefore, it is important to provide treatment and long-term follow-up examinations for patients with LP. Long term immunosuppressive or immunomodulatory agents may have to be prescribed as an important mode of treatment for OLP. Therefore specifically blocking the proteins involved in this disease process could in future reduce the dependency on steroids, thus reducing their side effects. Apoptotic inhibitors if used in general may affect the other normal physiologic process. So knowing the involvement of specific protein in apoptosis, in future may allow the use of peptide inhibitor, so as to interfere with the disease pathogenesis and its progression. This needs more such studies to support or clarify this hypothesis. By additional supportive studies directed at knowing the specific pathway involved in the disease pathogenesis, the study result can be utilized for the patient's benefit and thus reducing their sufferings.

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