Original Article

Survivin expression in oral lichen planus: Role in malignant transformation

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Abstract

Context: Oral lichen planus (OLP) is a potentially malignant disease with a prevalence rate of 0.5–2.2%. It is a T-cell-mediated autoimmune disease, in which cytotoxic CD8+ T-cells trigger apoptosis of the basal cells of oral epithelium. The reported progression of OLP to oral squamous cell carcinoma (OSCC) ranges from 0.4% to 6.5%. Apoptosis plays a major role in the maintenance of tissue homeostasis. The evasion of apoptosis in the form of dysregulation of inhibitors of apoptosis proteins (IAPs) may lead to malignant transformation. Survivin belongs to the second gene family of IAPs, which is overexpressed in many tumors such as OSCC and gastric carcinomas, and its expression is widely involved in apoptosis as well as in tumor metastasis.

Materials and Methods: Sections were obtained from the paraffin-embedded archival blocks of patients diagnosed histologically as OLP, and cases with normal epithelium were used for comparison whereas cases with OSCC were used as positive control.

Results: We analyzed the expression of survivin in OLP and normal epithelium. Survivin expression with moderate intensity was seen in the cells of basal layer with nuclear positivity in cases of OLP, whereas mild to nil expression was seen in normal epithelium with nuclear and cytoplasmic positivity in different layers.

Conclusions: Survivin positivity was seen predominantly in the basal cells of OLP suggesting increased longevity of these cells which in turn might acquire dysplastic changes leading to increased risk of malignant transformation of this premalignant condition. Although the conversion rate may be low, the potential exists in the indolent course of the disease.

Key Words: Anti-apoptotic proteins, malignant transformation, oral lichen planus, oral squamous cell carcinoma, survivin

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INTRODUCTION

Lichen planus is a chronic inflammatory mucocutaneous disease that affects the skin and mucosa. Its prevalence worldwide is about 1% to 2%, whereas among Indians, it was found to be around 1.5%.[1] Oral lichen planus (OLP) is a T-cell-mediated autoimmune disease that leads to the destruction of basal cell layer of oral mucosa.[2,3]

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The etiopathogenesis of OLP is highly controversial, which involves the specific and nonspecific mechanisms. Specific mechanisms include antigen presentation by basal layer of keratinocytes followed by death of these antigen-specific keratinocytes caused by cytotoxic T-lymphocytes. Nonspecific mechanisms are mast cell degranulation and matrix metalloproteinase activation.[4] These combined mechanisms

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cause T-lymphocyte accumulation in the lamina propria underlying the epithelium, rupture of the basement membrane and T-lymphocyte migration leading to apoptosis of keratinocytes, all of which are characteristic features of OLP.[5]

In OLP, the basal epithelial cells undergo apoptotic change termed as liquefactive degeneration which is due to the proximity of subepithelial lymphocytic infiltrate.[6] The basal epithelial cells of the oral mucosa are presumably attacked by the T-lymphocytes. The attacked cells trigger a series of complex molecular mechanisms designed to arrest the cell cycle for DNA repair, induce cell senescence, or induce apoptosis to eliminate cells with severely damaged DNA.[7]

Several studies have clinically reported OLP cases as having an increased risk of malignant transformation. Because of explicit clinical and histopathological criteria, the incidence of oral cancer in patients of OLP merits this mucosal disease, and hence, the WHO included OLP among the premalignant conditions.[8] The cause of increased oral cancer risk in OLP patients is unknown although the oral mucosa affected by OLP may be compromised to the extent of being more sensitive to exogenous mutagens in tobacco, alcohol, betel quid and *Candida albicans*.[1] Alternatively, the chronic inflammatory response and simultaneous epithelial wound healing response in OLP may increase the likelihood of cancer-forming gene mutations. These were the few hypotheses which have been proposed and the malignant transformation rate of OLP was found in the range of 0.4–5.6%.[9]

Survivin belongs to the second gene family of an inhibitor of apoptotic proteins which were recently identified.[10] It is overexpressed in many cancers but not in normal differentiated adult tissues.[11] The expression of survivin is widely involved in apoptosis, embryo development, blood vessel growth, immune regulation as well as tumor metastasis.[12]

In studies of oral squamous cell carcinoma (OSCC), the expression of survivin protein has been noted to play a vital role in oral carcinogenesis.[13] It is proposed that OLP lesions that are in the process of transforming to OSCC present histomorphologically as OLP but possess certain molecular signatures, that represents the specific factors that drive progression to cancer.[2]

It is accepted that the lesions identified as carcinomas may be preceded by premalignant conditions. However, this premalignant condition does not imply the development of a carcinoma. This brings up the necessity to search for biologic markers that enhance and clarify the carcinogenesis process, making it possible to identify some molecular alteration that results in the development of cancer, independent of morphologic change recognition. Thus, aiming to contribute to the identification of lesions that may present a premalignant character, the immunohistochemical expression of survivin in OLP was analyzed.

SUBJECTS AND METHODS

Sample

Seventy cases were selected from the archives of the Department of Oral and Maxillofacial Pathology. Cases were divided into three groups, Group 1/control group - 10 cases with stretches of normal mucosa, Group 2-50 cases diagnosed histopathologically as OLP and Group 3 -10 cases of OSCC (used as positive control). Lesions were diagnosed as OLP based on their clinical and histopathological features. The H and E-stained slides were reviewed to confirm histopathological diagnosis.

The diagnosis was reinforced by three pathologists in a blind trial.

Immunohistochemistry

All samples used in immunohistochemistry were fixed with 10% formalin and embedded in paraffin. Four-micrometer thick tissue sections were obtained, deparaffinized and rehydrated with graded alcohol series. The evaluation of survivin protein was made by the streptavidin-biotin-peroxidase method. For antigen retrieval, ethylene diamine tetra acetic acid buffer (pH 8.0) was used in a water-bath and was kept in a pressure cooker for 30 min. For blocking of endogenous peroxidase, the sections were incubated with hydrogen peroxide block for 20 min. Power block was then applied for 20 min, followed by primary antibody for 90 min and then washed with Tris wash buffer solution. After incubation with primary antibody (survivin), the sections were exposed to the secondary antibody for 30 min. Protein expression was developed with diaminobenzidine 0.03%–0.6 ml of hydrogen peroxide substrate complex and counterstained with Harris hematoxylin. Positive controls were included in all reactions.

Analysis

The quantitative analysis of positive cells for survivin was accomplished using morphometry under ×200; the epithelium of normal mucosa, OLP and OSCC was evaluated. Only cells that presented nuclear brown-colored staining were considered positive. The intensity of staining was considered comparing with positive controls and the layer in which positive cells were present was also considered.

The images of each field were obtained in a light microscope ×200, under a firm focus and with sharpness of field and then transferred to a TV monitor attached to a computer system, in which the manual counting of the nuclei that expressed the proteins was achieved after their individualization by ProgRes software (The ProgRes CapturePro microscopic camera software, Jenoptik).

This manual counting of positive cells was made by counting positively stained nuclei of the cells, approximately in a

stretch of epithelium with a length of 100 μm, the selected field for counting being randomly chosen. Kruskal–Wallis and Mann–Whitney test were applied to assess statistical differences between the group of lesions, and a value of $P < 0.05$ was considered statistically significant.

RESULTS

Cases of OLP, normal mucosa and OSCC were evaluated using survivin immunostaining in the epithelium. Nuclear dark-brown staining was considered as positive.

In OLP, out of 19,340 total cells that were counted in 100 μm length of epithelium for all the cases, 5144 cells were positive for survivin immunostaining [Figure 1]. The expression was seen mainly in the basal layer of cells (5105 cells) followed by spinous layer of cells (40 cells) [Figure 2] and all the cells showed nuclear positivity with moderate intensity.

In normal epithelium, out of 4632 total cells that were counted in 100 μm length of epithelium for all the cases, 9 cells were positive for survivin immunostaining [Figure 3]. The expression was seen mainly in basal layer of cells (9 cells), out of which four cells showed cytoplasmic positivity and five of which showed nuclear positivity of mild intensity.

In OSCC, out of 2023 total cells that were counted in 100 μm length of epithelium for all the cases, 1184 cells were positive for survivin immunostaining [Figure 4]. The expression was seen in different strata of the epithelium; mainly; it was observed in the middle one-third of the epithelium (1184 cells). All the cells showed intense nuclear staining.

Mean positive cells in the given layer of epithelium is as presented in Graph 1.

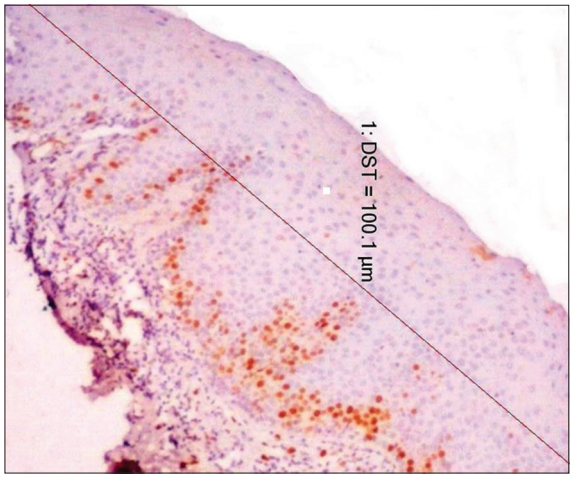


Figure 1: Photomicrograph of survivin immunostaining showing positivity in basal cells predominantly (IHC stain, ×100)

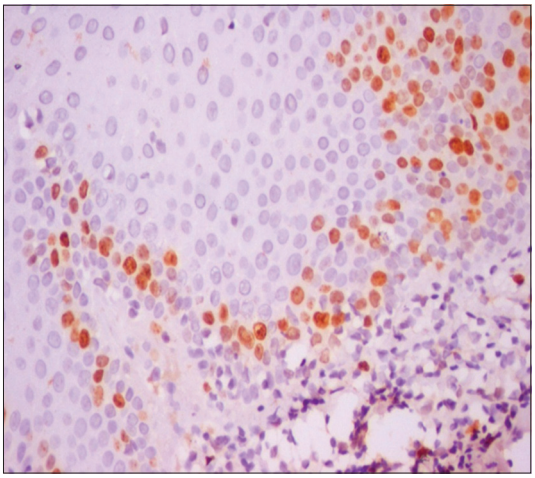


Figure 2: Photomicrograph of survivin immunostaining showing positivity in basal cells predominantly (IHC stain, ×200)

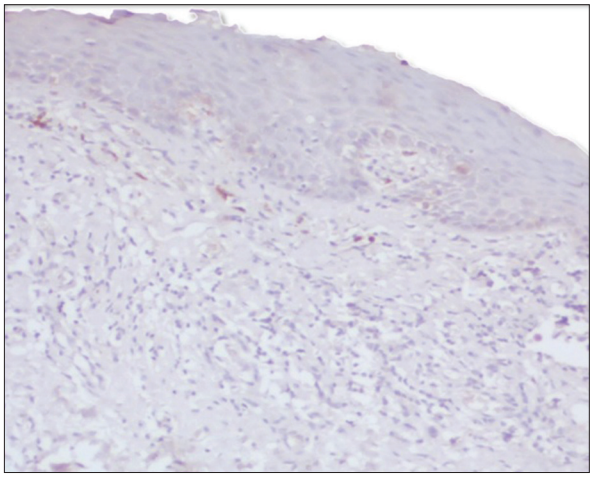


Figure 3: Photomicrograph of normal epithelium showing faint survivin immunopositivity in one of the basal cells of the epithelium (IHC stain, ×100)

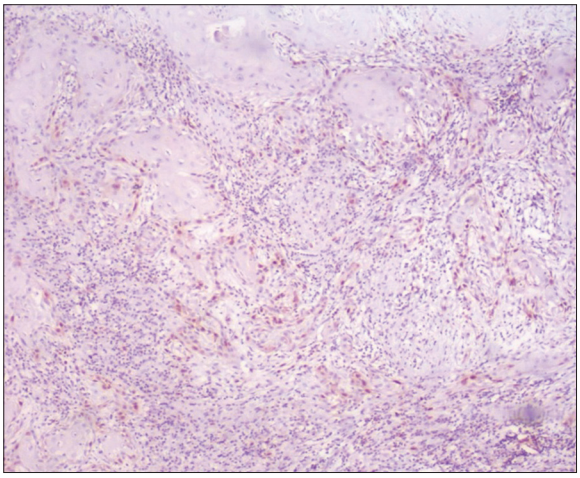
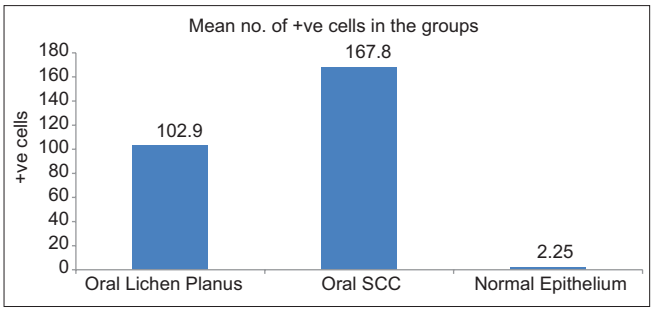


Figure 4: Photomicrograph of oral squamous cell carcinoma showing intense survivin immunopositivity in basal cell layer of the epithelium (IHC stain, ×100)



Graph 1: The mean positive cells seen in all the three study groups. Positivity in Oral lichen planus was about 102.90 which were close to oral squamous cell carcinoma (167.80), least was noted in normal epithelium (2.25). The difference between the staining in the two groups of lesions was statistically significant ($P < 0.001$)

The difference in mean positive cells among the groups was found to be statistically significant ($P < 0.01$) using Kruskal–Wallis test. To find out among which pair of groups, there exist a significant difference; multiple comparisons were carried out using Mann–Whitney test and the results obtained showed the difference in mean number of positive cells between OLP and normal epithelium was found to be statistically significant ($P < 0.01$).

DISCUSSION

OLP is a common mucocutaneous lesion which affects about 1–2% of general global population according to the WHO and about 1.5% of Indian population. Women are predominantly affected than men, the ratio being 1.4:1.[14] Clinically, OLP may present in the mouth in reticular, erosive, papular, plaque-like, atrophic or bullous form. Most commonly, it affects the buccal mucosa, tongue, followed by gingiva and floor of the mouth.[4]

Histologically, OLP is characterized mainly by hyperplastic or atrophic epithelium with hyperparakeratosis, apoptosis of basal layer of cells and dense band-like inflammatory infiltrate in the juxtaepithelial connective tissue. The infiltrate consists of lymphocytes, mainly T-lymphocytes.[15]

The etiopathogenesis of the disease is still unknown; several factors such as genetic background, infection, stress, immunodeficiencies, food allergies, trauma and habits have been proposed. Various mechanisms have been hypothesized to describe the immunopathogenesis of lichen planus which include antigen-specific cell-mediated immune response, nonspecific mechanisms, autoimmune response and humoral immunity. It is thought to be a T-cell-mediated autoimmune disease in which the autocytotoxic CD8+ T-cells trigger apoptosis of the basal epithelial cells.[5]

Although there are various treatment modalities, OLP lesions go into remission periods of either long or short duration. Thus, it has been postulated that chronic inflammation and simultaneous wound healing response in OLP may increase the likelihood of cancer forming gene mutations. This hypothesis is well supported by recent findings that link the T-cell-induced chemical mediators of inflammation to tumorigenesis.[16,17]

Alternatively, it has also been proposed that oral mucosa affected by OLP may be compromised to the extent of being more sensitive to exogenous mutagens such as tobacco, alcohol, betel quid, recurrent trauma and *C. albicans*. Thus, an interaction between various carcinogens including tobacco at clinical level with an altered epithelium of OLP at the histological level may lead to cancer development.[14]

It has been proposed that regulated on activation, normal T-cell expressed and secreted (RANTES), a chemokine expressed by T-cells in OLP infiltrate, can induce the expression of the phosphatidylinositol 3-kinase and Akt (protein kinase B) enzymes which can trigger a cascade of pro-proliferative and pro-survival transduction signals that may act on basal cells in OLP leading to proliferation of these cells which were about to undergo apoptosis. Increased longevity of these cells may lead to dysplastic changes which increase the risk of malignant transformation in cases of OLP.[18-20]

Survivin is a protein that inhibits apoptosis and regulates cell division. Survivin is expressed in embryonic tissues as well as in the majority of human cancers but is not expressed in most normal adult tissues including hyperplastic epithelium without associated dysplasia wherein 5–10% (not more than that) of cells may express survivin. The cancer-specific expression of survivin coupled with its importance in inhibiting cell death and regulating cell division marks survivin as a useful diagnostic marker that can predict a potency toward malignant transformation.[21,22]

Aberrations in apoptotic programs are a hallmark of perhaps all cancers that potentially affect various stages of malignant transformation. Since OLP is associated with apoptosis of basal cells, the study of survivin, an anti-apoptotic marker, may be useful to understand the premalignant nature of OLP and its potential to progress toward a malignant state.

In the present study, survivin was most commonly expressed in the basal layer of the epithelium in cases of OLP with strong nuclear positivity indicating the inhibition in apoptosis which might act as a molecular signature in malignant transformation.[2]

CONCLUSION

In our study, higher mean positive cells for survivin were recorded in oral SCC group (28.43, mean positive cells), followed by OLP group (16.65 mean positive cells) and normal epithelium

group (2.25% mean positive cells). Survivin expression was seen in 95% cases of OLP which was mainly of moderate intensity (46%), followed by mild intensity in 44% of cases. The expression was predominantly seen in the basal layer of cells (5104 cells) and all the cells showed nuclear positivity (100%).

Since expression of survivin was seen mainly in basal cells and the mean positive expression was higher than normal epithelium, it could be concluded that though the malignant transformation rate of OLP is considered to be low, it definitely has a potential for malignancy which cannot be overlooked. Based on a person's habit and environment of the tissue, the epithelium has a potency to convert into a dysplastic lesion which can finally cascade into frank invasive SCC.

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Conflicts of interest

There are no conflicts of interest.

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