Research Article

Immunohistochemical Evaluation of Ki-67 Expression in Erosive and Non-Erosive Oral Lichen Planus

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Abstract

Background: Oral lichen planus (OLP) is a relatively common, chronic inflammatory condition, which is considered a precancerous lesion. The Ki-67 antigen is expressed in all the phases of the cellular cycle in proliferative cells. Different studies have suggested the relationship between incidences of malignancy in precancerous lesions and the occurrence of this protein.

Objectives: This study aimed to evaluate Ki-67 expression in erosive and non-erosive oral lichen planus.

Materials and Methods: Specimens (formalin-fixed and paraffin-embedded) of 30 lesions of erosive OLP and 30 lesions of non-erosive OLP were referred for immunehistochemistry (IHC) analysis of Ki-67. Results of immunohistochemistry were statistically evaluated by means of the chi-square test and independent t-test. The level of statistical significance was established at $P < 0.05$.

Results: The mean expression of Ki-67 in patients with erosive OLP was higher than people in the control group. These differences were statistically significant ($P = 0.041$).

Conclusions: Since Ki-67 is extensively accepted as an important biomarker in diagnosis, prognosis and treatment of cancerous and precancerous lesions, a high degree of presence of this biomarker in chronic precancerous lesions, such as erosive OLP, can be of great use in prognosis and suggested treatments.

Keywords: Lichen Planus, Oral, Ki67, Immunohistochemistry

1. Introduction

Oral lichen planus (OLP) is a mucocutaneous disease with a chronic inflammatory process characterized by T-cell mediated immune response and mixed patterns of both apoptosis and increased cellular proliferation occurring simultaneously (1).

The world health organization (WHO) classifies OLP as a "potentially malignant disorder" with unspecified malignant transformation risk and suggests that OLP patients should be under close monitoring (2). Severe erosive disease, leaving mucosal atrophy and requiring systemic treatment, is reported to carry the highest risk of malignant transformation (3). Recently, OLP has been proposed as an ideal model of inflammation-induced cancer (4). Several molecular studies have shown evidence of increased cellular turnover rate, in the form of increased cellular proliferation, in epithelial cells of oral lichen planus (5-8).

Ki-67 is a nuclear protein that can be detected in all phases of the cell cycle [U+066B] except for the G0 phase.

This antigen is a proliferation marker that correlates with the presence and severity of epithelial dysplasia (9, 10). The expressions of Ki-67 have been examined in pre-malignant and malignant oral mucosal lesions predominantly from the West (9). However, the advances in molecular information on this pathologic condition have shed new light on the complex pathogenesis of OSCC (oral squamous cell carcinoma) arising in OLP.

2. Objectives

This study mainly focused on investigating the possible relationship between the clinical presentation and epithelial proliferative activity in oral lichen planus cases assessed by immunohistochemical expression of human Ki-67 protein.

3. Materials and Methods

A total of 60 patients with OLP (30 erosive OLP as the case group and 30 non-erosive OLP as the control group) were recruited in this descriptive-comparative study. This study was conducted at the clinic of oral medicine of Tabriz University of Medical Sciences in 2012 and approved by the ethics committee of Tabriz Medical University.

A clinical diagnosis of OLP was established when reticular or popular textures were present clinically and in the histopathologic appearance; meaning, when basal cell degeneration and/or infiltration of inflammatory cells, such as T lymphocytes, were observed (1).

The exclusion criteria were considered to be:

1) The presence of any factor related to the lichenoid reaction, such as amalgam fillings and crowns, near the lesion and consumption of medications that are associated with lichenoid reaction such as metformin and ACE inhibitors;

2) Patients with histopathologic findings in favor of dysplasia;

3) The presence of disinterest for being involved in the study;

4) Patients with systemic diseases, malignancies or dermal diseases that would influence the immune system.

The patients' records were completed and necessary examinations were performed. All specimens (formalin-fixed and paraffin-embedded) were cut with 4 mm thickness, and mounted on slides. For immunohistochemistry analysis, slides were placed in an oven with 56°C dry heat for 30 minutes for deparaffinization and then washed in alcohol and xylol solutions.

All the samples were pretreated with 0.3% H_2O_2 in 30% methanol for 60 minutes in order to have the endogenous peroxidase blocked. For antigen retrieval, the slides were placed in a citrate buffer at 95°C for five minutes. Then they were washed in phosphate buffered saline (PBS). For blocking the endogenous peroxidase, sections were incubated in 3% H_2O_2 for 10 minutes. The slides were placed in distilled water and PBS, then incubated with the primary antibody (Ki-67 antibody, Zymed, England; diluted, 1:100) for one hour and washed with PBS. In this stage, the slides were incubated with biotinylated secondary antibody for 10 minutes and washed with PBS. Then the slides were incubated with horseradish peroxidase (HRP) for 10 minutes and washed with PBS. Application of diaminobenzidine hydrochloride chromogen for 10 minutes and washing with tap water were performed with hematoxylin and rinsed in tap water and were mounted. For negative controls, the same procedure was carried out with normal serum instead of each antibody. As a positive control for Ki-67 immunostaining appendix tissue was also used. The quantitative analysis of cells positive for Ki-67 was performed by only one observer. Only cells that presented nuclear brown-colored staining were considered positive. The presence only was enough to distinguish positive from negative cells and the intensity of staining was not considered.

All of the slides were observed by light microscopy at X400 magnification, the selected field for counting being randomly chosen. Counting the percentage of positive nuclei in 400 consecutive epithelial cells of selected areas representative of the lesion gave a semi-quantitative evaluation of the immunohistochemical results. The quantitative analysis of cells positive for Ki-67 was accomplished by only one observer. Only cells that presented nuclear brown-colored staining were considered positive (11). It was only enough to distinguish positive from negative cells Ki-67 and the intensity of staining was not considered.

The obtained data were analyzed by statistical methods (chi-square test) using the statistical software SPSS 15, and for confirmation of the occurrence rate of Ki-67 and labeling index, independent t-tests were used. In this study, the level of statistical significance was established at P < 0.05.

4. Results

The present study evaluated 30 patients with erosive OLP and 30 patients with non-erosive OLP. Sixty five percent (65%) of tissue specimens were from the buccal mucosa, twenty percent (20%) from the tongue and fifteen percent (15%) from the palate and gums. As far as gender, 76.4% of patients were women and 23.6% were men. The mean age of patients was 44 years, while the maximum age was 86 and the minimum was 18 years.

Based on the data, 93.3% of patients with erosive lichen planus, show positive occurrences and 6.7% were negative. In patients with non-erosive lichen planus 75% occurrences were positive and 25% were negative (Table 1). Data were analyzed by statistical methods (chi-square test) with the statistical software SPSS 15 (P = 0.041). Results show that the average incidence of protein Ki-67 in erosive lichen planus is significantly more than in non-erosive lichen planus.

5. Discussion

In the present study, we examined the rate of Ki-67 expression in cases with erosive OLP and non-erosive OLP. Based on the immunohistochemical evaluations, the mean expression of Ki-67 in erosive lichen planus was significantly higher compared with non-erosive lichen

Table 1. Expression of Ki-67 in Erosive and Non-Erosive Oral Lichen Planus[a]

Variables	Positive Expression	Negative Expression
Erosive oral lichen planus	93.3	6.7
Non-erosive oral lichen planus	75	25

[a]Values are expressed as percentage.

Table 2. Analysis of the Ki-67 LI in Erosive and Non-Erosive OLP

Variables	Sample Size	Min	Max
Erosive oral lichen planus	30	33	462
Non-erosive oral lichen planus	30	26.29	1078

planus. According to our results, the immunohistochemical panel composed of Ki-67 is useful for confirming the presence of dysplastic changes in OLP.

Cell proliferation can be followed up by the expression of some cell proteins such as the Ki-67 antigen ([12]). Ki-67 is a nuclear antigen expressed during the G1, S, M and G2 periods of the cell cycle. The expression level of Ki-67 indicates the status of cell proliferation. The usefulness of the suprabasal expression of Ki-67 is as an objective marker for the presence of epithelial dysplasia of the oral mucosal; there is a greater frequency of suprabasal expression of Ki-67 as the severity of the dysplasia increases, as revealed by Gonzalez-Moles et al. ([13]). The findings of Gonzalez-Moles are supported by Li et al. who found that basal and superficial layers showed the clearest difference between the normal and abnormal tissues ([14]). In many studies including oral lesions, the reported incidence of Ki-67 expression has varied from 96% to over 100% ([8]).

Macluskey et al. showed a statistically significant correlation with mean of Ki-67 LI in healthy tissue, dysplasia, and in carcinomas, thereby suggesting that epithelial proliferation may continue to increase during the transition from dysplasia to carcinoma, but this is likely to occur at a slow rate ([15]). Our results confirm the normal immunohistochemical profile in normal epithelial mucosa.

Dwivedi et al. ([16]) confirmed that suprabasal expression of Ki-67 provides an objective criteria for determining the severity of dysplasia and histological grading of OSCC and can be used as an objective marker to evaluate the grading of epithelial dysplasia and OSCC.

Taniguchi et al. showed that the rate of Ki-67 expression in OLP was more than in normal mucosa. They considered Ki-67 expression as a marker of the dysplasia in the oral epithelium, stating that the proliferate status of the lesion and increased cell proliferation in OLP is likely to be a secondary phenomenon due to the damage inflicted on keratinocytes by infiltrating mononuclear cells in the submucosa ([6]). In Taniguchi's study most samples were from buccal mucosa and of the reticular type, whereas in our study the erosive type were also included ([6]).

Agha-Hosseini et al. ([11]) observed that the expression of a Ki-67 marker was significantly higher in 44 specimens with OLP in comparison with 30 controls. They also concluded that OLP should be regarded as a premalignant condition. Although our results are in agreement with this study, we have found that Ki-67 is the most constant marker associated with a pattern of lesions and in a higher percentage.

Pirkic et al. ([17]) showed that the Ki-67 LI in erosive OLP was severe and found to be associated with the degree of inflammation in lesions. In this study, 30 samples of erosive oral lichen were evaluated. In this study, the distribution of age, sex and location of the lesions had not been noted.

According to Neppelberg's study, 47% of oral lichen planus cases showed a severe rate of Ki-67 expression, and 27% showed an average incidence in comparison with normal mucosa. Although it was discussed that the Ki-67 protein was an important factor in determining the prognosis of cancer, in this study, this marker was not considered as a prognostic factor in oral lichen planus, and not introduced as a good marker in this regard ([18]).

In another study by Mattila et al. ([19]), the Ki-67 expression in oral lichen planus was not in the balance and in the atrophic oral lichen planus has limited value ([20]). It seem that the Ki-67 protein incidence was reduced in old tissue samples of oral lichen planus lesions because of low protein half-life in past studies. According to Hadzi-Mihailovic et al. study, Ki-67 was more expressed in keratinocytes and lymphocytes of OLP patients compared with HI, but less compared with patients with SCC ([20]).

To summarize, the immunohistochemical differences found between non-erosive OLP and erosive OLP support

the usefulness of the expression of Ki-67 as an objective marker for the presence of epithelial dysplasia of the oral mucosa and show a greater frequency of expression of Ki-67 as the severity of the dysplasia increases.

5.1. Conclusions

To conclude, Ki-67 expression is a proliferation marker in erosive OLP lesions and might have a predictive value in oral lichen planus lesions prone to developing malignancy. Studies with a larger sample size are needed to obtain a cut-off value for distinguishing between non-dysplastic and dysplastic oral epithelium using Ki-67 as an objective marker and to aid in early detection of oral premalignant and malignant lesions.

Acknowledgments

Footnotes

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