Immunohistochemical Study of p53 Expression in Patients with Erosive and Non-Erosive Oral Lichen Planus

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ABSTRACT

Statement of the Problem: Oral lichen planus is a common mucocutaneous lesion with a chronic inflammatory process mediated by immune factors while a few cases of the disease become malignant.

Purpose: This study aimed to determine the frequency of p53 marker as a tumor suppressor in patients with erosive and non-erosive oral lichen planus (OLP) by using immunohistochemical methods.

Materials and Method: This descriptive cross-sectional study investigated the p53 expression in 16 erosive OLP, 16 non-erosive OLP samples, and 8 samples of normal oral mucosa through immunohistochemistry. The percentage of stained cells in basal and suprabasal layers, and inflammatory infiltrate were graded according to the degree of staining; if 0%, <10%, 10-25%, and >50% of the cells were stained, they were considered as (-), (+), (++), (+++) and (++++), respectively. The obtained data was statistically analyzed and compared by using Chi square and Fisher's exact test.

Results: The mean percentage of p53 positive cells in erosive OLP (34.5±14.2) was considerably higher than that in non-erosive OLP (23.8±10.4) and normal mucosa (17.5±17). There was a significant difference among the three groups of erosive, non-erosive and control in terms of staining intensity. No significant difference existed between the patients' age and sex in the two OLP groups.

Conclusion: The increased incidence of p53 from normal mucosa to erosive OLP indicated the difference between biological behavior of erosive and non-erosive OLP. It can be claimed that the erosive OLP has great premalignant potential compared with the non-erosive one.

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Introduction

Oral lichen planus (OLP) is a common mucocutaneous lesion with chronic inflammatory progression that is presumably caused by activation of the immune response to skin or mucous changes. [1] The prevalence of this disease is 2% in the general population. [2] The etiology of lichen planus is still unknown; however, factors such as immunologic factors, genetics, medications, and hepatitis C can contribute to the pathogenesis of lichen planus. [3]

Studies reported the possible transformation of this lesion to a malignant complication in long term; therefore, WHO considered this lesion as a premalignant condition. However, there is skepticism and considerable uncertainty about the premalignancy of this lesion. [4] Oral lichen planus is presented as white striations, white plaque, and erosions, which affect the oral mucosa, while erosive and atrophic forms have the most

common malignant transformation. [5] Various carcinogenic percentages for oral lichen planus are reported. [6]

Transformation of normal epithelium to neoplastic epithelium is the result of different genetic mutations that lead to loss of control in the mechanism of apoptosis and the subsequent changes in the differentiation of cells. In addition to the higher survival rate caused by new genetic mutations, the increased mitotic activity changes the pattern of epithelial cell maturation. [6]

It was recently discovered that changes in the mechanism of cell division and apoptosis are essential for carcinogenesis. Changes in appearance, followed by the function of such proteins may be used as a marker in malignant lesions for changing a lesion to a malignant case. [6] p53 is a well-known tumor suppressor gene whose mutational inactivation is observed in many human cancers. A normal gene of p53 protein in a cell represses tumor, suppresses carcinogenesis, and prevents oncogenes proliferation activities. Cells with p53 gene have the ability to delay the cell cycle until the damaged DNA is repaired or to move the damaged cells toward cell death (apoptosis). [7]

Mutations in the p53 gene are the common cause of molecular damage in human malignancy that causes cell formation with stability and incomplete half-life. Immunohistochemical assessment of the mutant P53 expression in OLP can be considered as a risk factor in addition to other factors. [2] When the protein is mutated or absent, the cells replicate the destroyed DNA and the mutations increase. The p53 tumor suppressor mutation is the most common molecular defect in human malignancies including oral squamous cell carcinoma. [1] In a study on OLP in 2013, Oliveira *et al.* [2] reported the over expression of important proteins such p53 in relation with the regulatory mechanisms of apoptosis in OLP, suggesting that there was a favorable environment for malignant transformation. Although the p53 is an important positive predictor of the prognosis of OLP, [8] only few studies have evaluated and compared the immunostaining of p53 expression in erosive and non-erosive OLP. Thus, the present study aimed to assess the expression of P53 in patients with erosive and non-erosive OLP through immune histochemistry.

Materials and Method

In this retrospective cross-sectional descriptive survey, the samples were collected from the archive of multiple pathology laboratories in Sari (Mazandaran province, Iran). The sample size was chosen based on a study by Aghahosseini and Mirzaii, [1] and according to the formula of sample size and placement ratio. Accordingly, 40 samples including 16 blocks of erosive OLP, 16 blocks of non-erosive lichen planus, and 8 blocks of normal oral mucosa were studied. All the included samples had adequate lesions and good fixations, since those of low quality and improper fixation were excluded.

Two 4-μ thick tissue sections were prepared from each block for immunohistochemistry staining to determine the expression of p53. [9] The employed immunohistochemical staining kit was the NoVo Link Polymer detection system (RE7140-K 250T; NOVO CASTRA, Germany), which was an updated version of Biotin Labeled Streptavidin (LSAB). Mice monoclonal antibodies against human P53 [Clone Do-7 1 ml RTU] (Code: Ncl-p53-Do7) were used according to the manufacturer's instruction (NOVO CASTRA; Germany).

To block the endogenous peroxidase, the sections were placed in 3% H_2O_2 for 3 minutes and incubated with antibody for 1 hour. Then, they were rinsed with phosphate buffered saline (PBS). They were placed in the biotinylated secondary antibody solution for 10 minutes, and were then washed with PBS again. The samples were placed in horseradish peroxidase (HRP) for 10 minutes, rewashed with PBS, placed in diaminobenzidine hydrochloride chromogen for 10 minutes, and then washed again. Finally, the slides were concentrated in hematoxylin, washed with water, and mounted.

The stained slides were studied under 100X and 400X light microscope (Nikon; Japan). For p53 staining, colon adenocarcinoma tissue was used as the positive control. [10] Counting the percentage of positive cells in 500 consecutive epithelial cells from the selected areas of lesion gave semi-quantitative assessment of the immunohistochemical results. [9]

The percentage of stained cells in the basal layer, suprabasal layers and inflammatory infiltration was graded according to the degree of staining as follows; 0% stained cells was graded as (-), <10% (+), 10-25% (++), 26-50% (+++), and if more than 50% cell were

stained it was considered (++++). The obtained results were compared and analyzed. The nuclear and cytoplasmic staining intensity by indicator were categorized in four groups including colorless or absence of any color (-), weak or light brown (+), moderate or chestnut brown (++), and severe or dark brown (+++). [10]

By employing SPSS software version 15, the data were descriptively analyzed (frequency-percent). Moreover, Chi square test was used to compare the study groups regarding the expression of P53. Furthermore, Fisher's exact test was used to determine the significance of differences among the groups.

Results

This study was performed on 32 samples of OLP (16 erosive and 16 non-erosive) and 8 blocks of normal mucosa samples. Out of 32 OLP patients, 17 were females (53.1%) and 15 were males (46.9%) with the mean age of 46 ± 0.81 years old. There was no significant correlation between the presence of OLP and age and sex in both erosive and non-erosive groups.

The most frequently involved area in erosive OLP group was the gingivae (50%), followed by buccal mucosa (31%) and labial mucus (19%). In non-erosive OLP group, the buccal mucosa (56%), gingivae (31%), and labial mucosa and floor of the mouth (6% each), respectively, accounted for the most affected areas. Accordingly, the percentage of p53 expression revealed a significant different between the number of cells stained in the three groups (erosive, non-erosive, and control) (Table 1).

**Table 1:** Frequency distribution of stained cells in each study group based on index of positivity

Study groups	Sample Size	0	1-10 N (%)	10-25 N (%)	26-50 N (%)	50> N (%)
Non-erosive	16	0 (0%)	9 (56%)	7 (44%)	0 (0%)	0 (0%)
Erosive	16	0 (0%)	1 (6%)	7 (44%)	8 (50%)	0 (0%)
Normal mucosa	8	5 (62%)	3 (38%)	0 (0%)	0 (0%)	0 (0%)

The mean percentage of p53 positive cells in erosive OLP (34.5 ± 14.2) was considerably higher than that in non-erosive OLP (23.8 ± 10.4) and normal mucosa (17.5 ± 17). Figures 1-3 display some expression patterns. The number of stained cells revealed significant differences among the study groups regarding the expre-
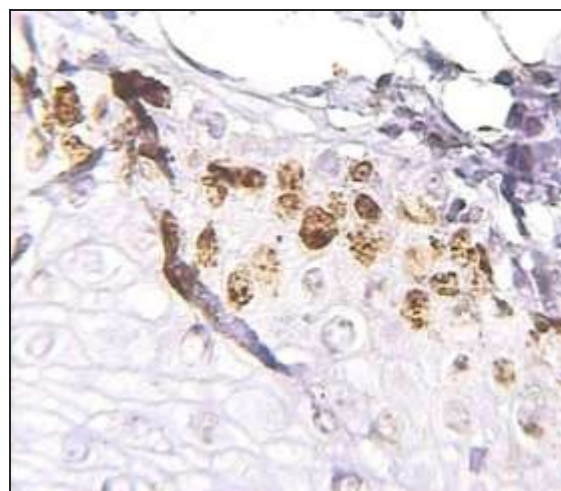
ssion of P53 (Table2) (Figure 1, 2 and 3).

Figure 1: Nuclear expression of P53 in non-erosive oral lichen planus in basal layer with 25% cellularity and light brown intense staining (×100)
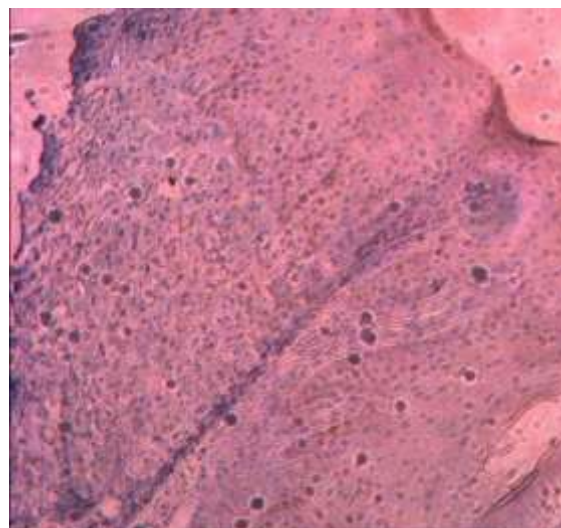
Figure 2: Nuclear expression of P53 in erosive oral lichen planus in basal layer with 40% cellularity and chestnut brown intense staining (×100)

Figure 3: Nuclear expression of P53 in erosive oral lichen planus in basal layer with 50% cellularity and dark brown intense staining (×400)

Table 2: Mean SD and comparison of expression of P53 based on the number of stained cells in study groups

Study groups Markers	OLP(Erosive)			OLP(Non-erosive)			Normal mucosa			p Value
P53	Total	Mean	SD	Total	Mean	SD	Total	Mean	SD	
	16	33.5	14.2	16	22.8	10.4	16	1.75	17	‹0.05

Table 3: The quantitative intensity expression of P53 in study group

Study groups	Sample Size	Colorless (-)	Light brown (+)	Chestnut Brown (++)	Dark brown (+++)	p Value
Non-erosive	16	0 (0%)	12 (75%)	6 (37.5%)	0 (0%)	
Erosive	16	0 (0%)	2 (12.5%)	10 (62.5%)	4 (25%)	‹0.00
Normal mucosa	8	6 (75%)	2 (25%)	0 (0%)	0 (0%)	

As presented in Table 3, the highest and lowest mean expression of p53 was respectively observed in the erosive OLP and the control group. There were significant differences between the two study groups and the control group. The erosive and non-erosive groups were not significantly different in in suprabasal and inflammatory infiltration locations; however, the groups were significantly different in basal location (Table 4).

Table 4: Frequency of the affected area in the two groups of oral lichen planus

Study groups Type of involvement	Non-erosive	Erosive	p Value
Basal	9 (56%)	5 (31%)	0.054
Suprabasal	4 (25%)	6 (38%)	0.63
Inflammatory infiltration	3 (19%)	5 (31%)	0.55

Discussion

Oral lichen planus is a mucocutaneous disease with a chronic inflammatory process characterized by T-Cell-mediated immune responses and mixed patterns of both apoptosis and increased cellular proliferation which occur simultaneously. [11-16] Since the first case of squamous cell carcinoma was developed from a mucosal lichen planus, the true odds ratio of such transformation is a matter of discussion. In general, different proportions of malignant potential of OLP, ranging from 0.04% to 1.74% is reported in literature. [17]

It is stated that p53 plays a key role in controlling the cycle, cell differentiation, and apoptosis. [18] It is also a valuable biomarker to predict malignant transformation in premalignant oral lesions. [19] The mutation of p53 tumor suppressor gene is the most common genetic disorder ever observed in human cancers. [20] Alterations in the expression of proteins related to the regulation of apoptosis (P53) can be used as markers of potential malignant transformation of epithelial lesions such as oral lichen planus lesions, suggesting close monitoring of OLP patients. [2, 20-21]

The present study examined the p53 expression intensity and percentage in cases with erosive and non-erosive OLP. Immunohistochemical evaluations revealed that the mean expression of p53 in erosive lichen planus was significantly higher than that in non-erosive lesions. Based on the current results, the immunohistochemical panel composed of p53 could help confirming any potential malignant change in OLP. The linear increase observed in the expression of markers from normal mucosa to erosive oral lichen planus indicated the difference of biological behavior between erosive and non-erosive OLP.

In a study by Eleni *et al.* [16] about the correlation between p53 and lichen planus prognosis, a statistically direct correlation was observed between the p53 incidence and the clinical characteristics of lichen planus. Moreover, more malignant changes were observed in lichen planus, as shown by p53 staining. [18-19] This was consistent with the results of the present study.

Aghahosseini and Mirzaii [1] demonstrated that unstimulated salivary p53 values in reticular OLP patients were significantly higher than that in healthy subjects and erosive forms. They concluded that plaque in the form of OLP was important in terms of its potential for malignancy and was not a safe form. [1] A different study by Seyedmajidi *et al.* [9] demonstrated that no significant difference between p53 and P63 markers in the two groups of erosive and reticular lichen planus. It was consistent with Montenbugnoli *et al.*'s study, [3] that reported no significant difference in p53 expression between the erosive and reticular lichen planus.

The current study found the gingivae (50%) and buccal mucosa (31%) to be the most commonly involved areas in erosive OLP; whereas, the buccal mucosa (56%) and gingivae (31%) were the most affected areas in non-erosive samples. In a study by Aghahosseini *et al.*, [17] the buccal mucosa (43.2%) was the

most commonly affected site. In a study, all samples were obtained from the buccal mucosa. [20] Other studies did not explicit the locations of OLP lesions. [17, 23-24]

The present study also detected the three groups to be significantly different regarding the color intensity of p53 marker. The color intensity was moderate in most samples of erosive OLP group, weak in most samples of non-erosive OLP group. In the normal group, most of the samples did not express the marker. None of the reviewed studies assessed the color intensity of p53 marker.

In this study, the frequency distribution of the affected area in two groups of erosive and non-erosive was examined under a microscope in terms of involvement in basal, suprabasal, and inflammatory infiltration. The obtained P-value did not show a significant difference between the groups. However, Varma *et al.* [25] reported that p53 could be used as a prognostic marker in premalignant lesion. They also showed that this was presented in the basal and suprabasal layers. [25] The results of this study was in contrast with the results of Gonzalez *et al.*'s study[26] on lichen planus which reported higher expression of p53 in the basal layer than in suprabasal and inflammatory infiltrate. Nor was it in line with Acay *et al.*'s [27] findings which showed the expression of p53 exclusively in basal and suprabasal layers in lichen planus and oral lichenoid lesions. In a study by Kövesi and Szende, [28] the severity of dysplasia, positivity and intracellular localization of (mutant) p53 expression increased based on the clinical form of leukoplakia which, similar to our study, indicated the increasing trend of P53 expression from normal mucosa towards non-erosive, and ultimately erosive lichen planus.

Analyses of immunohistochemistry and molecular biology in neoplastic and paraneoplastic lesions originated from the oral mucosa have shown that changes in tumor suppressor genes such as p53 may play an important role in oral carcinogenesis and they are potentially useful prognostic indicators. [14, 16] Studies with a larger sample size are needed to obtain a cut-off value for distinguishing the erosive and non-erosive oral epithelium by using p53 as an objective marker and to help the early detection of oral premalignant and malignant lesions.

Conclusion

Since p53 is extensively accepted as an important biomarker for diagnosis, prognosis, and treatment of malignant and premalignant lesions, high expression of this marker is useful for the identification of erosive OLP lesion with a more aggressive pattern and with a major tendency to malignancy.

Acknowledgment

Conflict of Interests

The authors declare no conflict of interests.

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