



## Comparison of Expression of p53 and bcl-2 Markers in Oral Lichen Planus and Oral Squamous Cell Carcinoma

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### ABSTRACT

**Background and Aim:** It has been suggested that oral lichen planus (OLP) can be a potential premalignant lesion. Reports are also available on the role of bcl-2 and p53 proteins in malignant transformation of OLP into oral squamous cell carcinoma (OSCC). Due to controversies in these reports, the present study aimed to compare the expression of p53 and bcl-2 markers in OLP and OSCC.

**Materials and Methods:** In this experimental study, immunohistochemical (IHC) staining was performed on erosive OLP and well-differentiated OSCC samples using bcl-2 and p53 antibodies. One-thousand cells per slide were counted, and the results were reported as percentages. The results were scored, and Mann-Whitney U test was used to compare staining grades in the two lesions.

**Results:** Twenty-two OLP and 25 OSCC samples with a mean age of 49.3±15.8 years were evaluated. Bcl-2 expression was reported to be 16.27±8.95% in OLP and 16.4±22.9% in OSCC. Expression of p53 was 30.86±28.26% in OLP and 49.6±29.6% in OSCC. The difference in bcl-2 expression between the two lesions was not significant (P=0.266), whereas this difference was significant for p53 expression (P=0.02). The correlation coefficient between these two markers was reported to be 0.45 in OSCC and 0.1 in OLP.

**Conclusion:** According to the results, there was a significant difference in p53 expression between OLP and OSCC samples, whereas this difference was not significant for bcl-2. There was no significant association between the expressions of these two markers in the samples. Therefore, there does not seem to be a high malignancy potential for the studied OLP samples.

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## Introduction:

According to available information sources, squamous cell carcinoma (SCC) comprises more than 90% of oral cancers, and the incidence of this disease in men is often greater than that in women.<sup>(1)</sup> On the other hand, lichen planus (LP) is a relatively common and chronic cutaneous disease that affects the oral mucosa and is present in about 1-2% of the adult population; however, despite extensive studies, the pathogenicity of LP is still unknown.<sup>(2)</sup> When the first case was reported in 1910, several studies suggested that patients with LP are at risk of developing cancer.<sup>(3)</sup> The most common form of oral LP (OLP) is the reticular subtype, and there is a possibility of dysplastic changes and malignant transformation (SCC) in erosive and atrophic subtypes.<sup>(4,5)</sup> The percentage of the incidence of OLP in the world varies from 0.5% to 2.6%, so that in various studies, depending on the patient's follow-up period, the percentage of malignant transformation has been reported to be between 0.5% and 10%.<sup>(6,7)</sup> In a research by Irani and colleagues in Iran, dysplastic changes were reported to be 10.7%.<sup>(7)</sup>

Balance between the molecules that control cell survival, such as bcl-2, and cell death, such as p53, is important in cell proliferation. Bcl-2 is an anti-apoptotic molecule found in the nucleus and mitochondrial membrane. This molecule has an inverse relationship with p53, so that its expression inhibits cell death due to apoptosis.<sup>(8)</sup> On the other hand, TP53 gene is a tumor suppressor gene and one of the most commonly mutated genes in human cancers. By means of correlated mechanisms, p53 neutralizes malignant transformation, initially by temporary activation of cell cycle arrest and then by inducing a permanent cell cycle arrest. Until recently, it was thought that p53 functions only through activating the transcription of aging, apoptotic, and anti-proliferation genes, and if DNA damage could not be restored, p53 induced aging or apoptosis.<sup>(8)</sup> P53 exerts its apoptotic function by expressing proteins involved in apoptosis, including the bcl-2 family.<sup>(8)</sup> The importance of these proteins in cell death and apoptosis has been investigated in

some oral lesions including oral SCC (OSCC), leukoplakia, oral dysplasia, and OLP.<sup>(8-11)</sup> Some studies suggest that OLP can be a potentially premalignant lesion, and both bcl-2 and p53 proteins can contribute to malignant transformation of OLP into OSCC.<sup>(12-16)</sup> So far, the expression of each of the mentioned proteins in these lesions has not yet been clearly demonstrated, and there are contradictions in this regard in the related studies.<sup>(17-22)</sup>

Considering the importance of early diagnosis and treatment of OSCC and in order to prevent or at least reduce the likelihood of recurrence and malignant changes in OLP, the present study aimed to compare the expression of p53 and bcl-2 markers in OLP and OSCC samples submitted to the Oral Pathology Department of the Dental Branch of Islamic Azad University of Tehran during 2006-2017.

## Materials and Methods:

In this experimental study, paraffin blocks related to patients with OLP and OSCC who referred to the Oral Pathology Department of the Dental Branch of Islamic Azad University of Tehran during the years 2006-2017 were examined. Samples with a definitive microscopic diagnosis of the lesions, with correct tissue fixation, with adequate tissue for staining, and without necrosis or excessive hemorrhage were included in the study. In order to match the samples and to eliminate the intervening variables, all OSCC specimens were well-differentiated, and all OLP specimens were of erosive subtype. These specimens were reviewed twice by an oral pathologist and were included in the study after reconsideration. Samples with necrosis, insufficient data or indefinite diagnosis were excluded from the study.

Using similar studies<sup>(3)</sup> and with the help of the analysis option for power and sample size for two proportions in Minitab software, considering  $\alpha=0.05$ ,  $\beta=0.2$ ,  $p1=0.4$ , and  $p2=0.7$ , the minimum required sample size in each group was estimated to be 25 samples.

For immunohistochemical (IHC) staining, 5- $\mu$ m sections were prepared and then stained with bcl-2 and p53 antibodies using the following procedure:

The sections were dewaxed, and each slice was soaked in 10 cc of citrate buffer (pH=6) and dried in a microwave for 10 minutes to retrieve the antigen.

The slices were placed in 0.3% liquid hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 minutes at room temperature. The sections were washed in phosphate-buffered saline (PBS) for 5 minutes and were adjoined with horse serum for 20 minutes in monoclonal antibodies against p53 and Pcl-2 protein (mouse clone 124; Dako, Denmark).

After washing in PBS, two to three drops of the secondary antibody (Elite, pk-6102) were added. The sections were incubated at room temperature, and after two rinses in PBS, all sections were stained with Mayer's hematoxylin, counted, and mounted. Additional sections were prepared using the parallel method with the removal of the primary antibody as negative controls; <sup>(1)</sup> breast cancer tissues were used as positive controls for p53, and normal pharyngeal mucosa was used as a positive control for bcl-2.<sup>(23,24)</sup>

To determine the expression of p53 and bcl-2, the tissue sections of the specimens were evaluated under a light microscope (Nikon, Japan) at 400 $\times$  magnification by two oral pathologist observers.

One-thousand cells were counted in a random field for each sample, and the mean of the results was reported as a percentage. Scoring was performed for each sample as follows: <sup>(3)</sup>

Score 0: staining of less than 5% of cells

Score 1: staining of 5% to less than 25% of cells

Score 2: staining of 25% to less than 50% of cells

Score 3: staining of more than 50% of cells

Mann-Whitney U test at a significance level of less than 0.05 was used to compare staining degrees in the two lesions. Also, the correlation between the expressions of the two markers was evaluated using Spearman's correlation coefficient. Figures 1 to 4 show the expression of p53 and bcl-2 markers in OLP and OSCC microscop-



**Figure 1: Expression of p53 marker in oral lichen planus (OLP) at 200 $\times$  magnification**



**Figure 2: Expression of p53 marker in oral squamous cell carcinoma (OSCC) at 200 $\times$  magnification**



**Figure 3: Expression of bcl-2 marker in oral lichen planus (OLP) at 200 $\times$  magnification**



**Figure 4: Expression of bcl-2 marker in oral squamous cell carcinoma (OSCC) at 400 $\times$  magnification**

**Result:**

In the current study, 47 samples were evaluated, including 22 OLP samples and 25 OSCC samples. OLP patients included 7 (31.8%) females and 15 (68.2%) males with a mean age of 49.3±15.8 years. OSCC patients included 11 (44%)

females and 14 (56%) males with a mean age of 67.5±12.9 years. Table 1 shows the frequency of bcl-2 and p53 expressions separately in OSCC and OLP samples.

**Table 1: Frequency of bcl-2 and p53 expression in oral squamous cell carcinoma (OSCC) and oral lichen planus (OLP)**

	Lesion	Mean±SD	P-value
Bcl-2	OLP	16.27±8.95	0.266
	OSCC	16.4±22.9	
P53	OLP	30.86±26.0	0.02
	OSCC	49.6±29.7	

SD=Standard Deviation

T-test showed that there was no significant difference in the expression of bcl-2 between OLP and OSCC samples (P=0.266), whereas this difference was significant for p53 expression between the samples (P=0.02).

**Table 2: P53 expression rate based on scoring**

Lesion	Score				P-value
	0	1	2	3	
OLP	2(9.1%)	10(45.5)	3(13.7%)	7(31.8%)	0.321
OSCC	0(0%)	5(20%)	9(36%)	11(44%)	

In evaluating the expression of p53 marker in OLP, 2 samples with negative expression (score 0), 10 samples with weak expression (score 1), 3 samples with moderate expression (score 2), and 7 samples with severe expression (score 3) were observed. In evaluating the expression of this marker in OSCC, 5 samples with weak expression (score 1), 9 samples with moderate expression (score 2), and 11 samples with severe expression (score 3) were observed; this indicates that p53 has a weak expression in patients with OLP in half of the cases, while in patients with OSCC, moderate and severe expressions are more frequently observed (Table 2).

**Table 3: Bcl-2 expression rate based on scoring**

Lesion	Score				P-value
	0	1	2	3	
OLP	4(18.2%)	14(63.6%)	3(13.6%)	1(4.5%)	0.055
OSCC	10(41.7%)	10(37.5%)	2(8.3%)	3(12.5%)	

In evaluating the expression of bcl-2 marker in OLP, 4 samples with negative expression (score 0), 14 samples with weak expression (score 1), 3 samples with moderate expression (score 2), and 1 sample with severe expression

(score 3) were observed. The expression of this marker in OSCC was as follows: 10 cases with negative expression (score 0), 10 cases with weak expression (score 1), 2 cases with moderate expression (score 2), and 3 cases with severe expression (score 3; Table 3).

**Table 4: Frequency of oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC) in different locations**

Location Lesion	Buccal mucosa	Upper lip	Tongue	Attached gingiva	Mandible	Alveolar ridge	Maxilla	Total
OLP	15(68.2%)	3(13.6%)	2(9.1%)	1(4.5%)	1(4.5%)	0(0%)	0(0%)	22(100%)
OSCC	4(16.0%)	0(0%)	11(44%)	0(0%)	3(12%)	3(12%)	4(16%)	25(100%)

The prevalence of OLP was 68.2% in the buccal mucosa, 13.6% in the lips, 9.1% in the tongue, 4.5% in the attached gingiva, and 4.5% in the mandible. The prevalence of OSCC was 16% in the buccal mucosa, 44% in the tongue, 12% in the mandible, 12% in the alveolar ridge, and 16% in the maxilla (Table 4).

There was no significant correlation between the expression of bcl-2 in OLP and OSCC samples with gender (P=0.943), age (P=0.867), and the location of the lesion (P=0.762), but regarding p53 expression in OLP and OSCC samples by gender (P=0.175), age (P=0.325), and the location of the lesion (P=0.042), it was found that only the site of the lesion was effective, which is the buccal mucosa, and does not have a significant relationship with age or gender. Moreover, the correlation coefficient between p53 and bcl-2 was 0.1 in OLP and 0.45 in OSCC.

**Discussion:**

LP is a relatively common chronic mucocutaneous disease that also affects the oral mucosa.<sup>(2,3)</sup> Some studies have suggested that OLP can be a potentially premalignant lesion, and both bcl-2 and p53 proteins can contribute to malignant transformation of OLP into OSCC.<sup>(12-14)</sup>

In the present study, expression of bcl-2 in OLP and OSCC was 16.27% and 16.4%, respectively, and this expression was mostly weak in OLP and mostly negative and weak

in OSCC. P53 expression in OLP and OSCC was 30.86% and 49.6%, respectively. P53 expression was mostly weak (45.5%) and severe (31.8%) in OLP and mostly moderate and severe in OSCC. This study showed that bcl-2 expression was not significantly different in the two diseases, but p53 expression in OSCC was more severe than that in OLP.

Leyva-Huerta et al examined the expression of p53 and bcl-2 in 37 patients (21 patients with OLP and OSCC and 5 with healthy gingivae). Twenty-one out of the 37 samples had OLP, and the ulcers were mostly located in the buccal region.<sup>(1)</sup> Higher incidence of OLP ulcers in the buccal mucosa was in line with the current research. In the study by Leyva-Huerta et al, p53 expression in OLP was mostly negative and mild, but the expression of p53 in five OSCC samples was, in most cases, severe and mild. Bcl-2 expression was negative in OSCC and OLP samples.<sup>(1)</sup>

The results of the study by Leyva-Huerta et al, similar to the current study, showed a significant difference in the expression of p53 between the two groups, and the expression of this factor in OSCC patients was higher than that in OLP patients. In comparison with the cited study, the current study suggests a more severe expression of p53 and bcl-2 markers. But the expression of p53 in the present study is less than that reported by Leyva-Huerta and colleagues.<sup>(1)</sup> This difference in the statistics can be due to the non-oral source of some of the samples in the cited research, and

this can be considered as an intervening factor.

A study by de Sousa et al, entitled “comparative analysis of the expression of proliferating cell nuclear antigen, p53, bax, and bcl-2 in OLP and OSCC”, showed that p53 expression in OLP and OSCC was 41.7% and 66.7%, respectively, and the expressions of bcl-2 in OLP and OSCC were both 16.7%.<sup>(4)</sup>

The expression of bcl-2 in both diseases in the cited study was similar to that reported in the current study; the expression of p53, according to the results of the current study, was higher in OSCC patients, but the percentage of expression in the study by de Sousa and colleagues is higher.<sup>(4)</sup> The reason for higher percentages, especially for OLP, is the use of different subtypes of LP in the research of de Sousa et al, while in the current research, erosive OLP was evaluated.

Nafarzadeh et al evaluated bcl-2 and bax expression in OSCC and OLP patients by examining 15 samples of erosive OLP, 15 samples of reticular OLP, and 11 samples of WOSCC (well-differentiated OSCC).<sup>(23)</sup> Bcl-2 was not observed in any of the OLP samples, while this marker was observed in 4 (36.4%) WOSCC samples.<sup>(23)</sup> Compared to the current study, the expression level of bcl-2 in WOSCC is higher in the cited research, and the lack of expression of bcl-2 in OLP is naturally lower compared to our research. This difference regarding WOSCC can be due to a small number of samples in the study by Nafarzadeh et al, especially for OSCC, that results in poor accuracy. Another reason for the discrepancy is the evaluation of WOSCC and only two types of OLP in the research by Nafarzadeh et al. Failure to observe bcl-2 in OLP samples is similar to the study by Leyva-Huerta and colleagues.<sup>(1)</sup>

de Sousa et al evaluated the immunohistochemical expression of bcl2, bax, p53, and PCNA in 24 cases of LP and 24 cases of OSCC. Expression of p53 was 41.67% and 66.67% in OLP and SCC, respectively, and expression of bcl-2 in both OLP and SCC was 16.67%.<sup>(3)</sup> Expression of bcl-2 in both diseases was similar to that observed in the present study, while regarding the expression of p53, the ratio of expression was similar in the study by de Sousa et al and the current research, but the rate of the expression of this protein in the research by de Sousa and colleagues is higher than that in the current study, which can be due to differences in tissue origin.

In a study by Stoicanescu et al, evaluating the role of p53 protein and Her-2/neu gene in 116 OSCC patients, expression of p53 was observed in 66.3% of the samples.<sup>(24)</sup> Compared to the current study, p53 expression levels were slightly higher. This difference can be attributed to the fact that in the current research, only OSCC samples of high degree of differentiation have been assessed. The researchers also concluded that the area with the highest incidence of cancer was the lower lip (22.4%) followed by the tongue (19.82%) and the floor of the mouth (18.96%), (24) while in the current study, the tongue (44%) followed by the buccal mucosa (16%) showed the highest prevalence of OSCC.

In the study by Crosthwaite et al, entitled “p53 protein expression in malignant, pre-malignant and non-malignant lesions of the lip”, four types of lip and oral lesions, including OSCC and OLP, have been considered.<sup>(25)</sup> In the cited study, p53 expression was positive in all OSCC cases, and no cases of lack of p53 expression in LP were found. In the mentioned study, p53 expression in OSCC is much higher than that reported in the present study, while its expression in LP is lower. This difference can be due to a small number of data, which reduces the accuracy of statistical analysis and the results.

The results obtained in this study are comparable to the results of similar studies, such as those performed by Leyva-Huerta et al,<sup>(1)</sup> Warnakulasuriya and Johnson,<sup>(26)</sup> and Schoelch et al,<sup>(27)</sup> all of which have reported a significant difference in the expression of this marker between OLP and OSCC. However, in the study by de Sousa et al, the difference in the expression of this marker was not significant in the two groups, and the researchers concluded this lack of difference as evidence for the absence of potential malignancy in OLP.<sup>(4)</sup> Of course, the difference in the number of samples and the method of IHC staining used to evaluate this marker could be among the reasons that justify the contradictions in the obtained results.

In the present study, no significant difference was observed in the expression of bcl-2 between the studied groups, in line with previous studies, including the studies by de Sousa et al,<sup>(4)</sup> Nafarzadeh et al,<sup>(23)</sup> and Leyva-Huerta et al.<sup>(1)</sup> Also, in the present study, there was no strong correlation

between the two markers in these samples, which is in line with the study by de Sousa et al who also reported the same result and considered the lack of correlation between these two markers as a reason for the absence of malignant potential in LP samples.<sup>(4)</sup>

The conversion of normal epithelium to neoplasm is the result of a series of genetic mutations that result in loss of cell control and apoptosis mechanisms, leading to a change in cell differentiation. This phenomenon increases the mitotic activity and consequently increases the cell survival, which itself is a suitable bedding for accumulation of genetic mutations and consequently a change in the pattern of maturation of the epithelial cells. Therefore, changes in the expression of contributing proteins in this mechanism are essential in the early stages of cancer development and may be considered as evidence of the potential for malignant transformation of a lesion.<sup>(3)</sup>

In this regard, p53 protein has a fundamental role in maintaining the stability of the cell's genome, because during apoptosis, it results in the flow of mechanisms related to DNA repair and the removal of damaged cells. In many studies, p53 mutation is considered essential for the initial phase of oral cancers.<sup>(28-32)</sup> For example, in a study by Valente et al, p53 histochemical analysis has been introduced as a useful tool in the evaluation and isolation of OLP samples that have more potential for malignant transformation.<sup>(32)</sup> Also, increased expression of bcl-2 makes it difficult to remove genetically modified cells and provides a suitable substrate for new mutations that ultimately lead to malignant phenotype. Bcl-2 has the potential to discontinue apoptosis in both the early and final stages of cancer, and the association between the expression of p53 and bcl-2 in pathologic lesions, including OLP, may be a significant evidence of their malignant transformation potential as a direct and positive correlation between this relationship and the degree of dysplasia of the oral mucosa has also been reported.<sup>(31)</sup>

Therefore, considering the significant difference in the expression of p53 between OLP and OSCC and failure to report a strong association between the expressions of these two markers in the present study, it seems that there is no high potential for malignant transformation of OLP. Further research on other apoptotic markers is also recommended.

## Conclusion:

In the present study, there was a significant difference in p53 expression between OLP and OSCC samples, whereas this difference was not significant for bcl-2 expression. There was also no strong association between the expressions of these two markers in the studied samples. Therefore, there does not seem to be a high malignancy potential for OLP samples examined in this study.

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