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Morphologic Evaluation of P53 Apoptotic Signaling Responses and Proliferative Activity of Ki-67 in Oral Lichen Planus, Oral Squamous Cell Carcinoma and Normal Specimens

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Oral lichen planus is not a rare condition in human being. This inflammatory disease is generally regarded as a benign pathology; however, transformation toward malignant conditions in some cases has forced many physicians to consider lichen planus as a premalignant entity. This study investigates and compares the rate of Ki-67 and P53 expression in patients with oral lichen planus and oral Squamous Cell Carcinoma (SCC) in comparison with normal subjects. In this prospective study, paraffin-embedded specimens of histopathologically proved oral lichen planus (n = 30), oral SCC (n = 20) and oral buccal mucosa of normal subjects (n = 20) were examined at Tabriz Imam Reza Hospital within a one-year period of time. Monoclonal antibodies against Ki-67 and P53 were used for determining the rate of expression of these two markers. The three groups were matched for patients' age and gender. Ki-67 was expressed in 26 patients (86.7%) in lichen planus group, in 20 patients (100%) in SCC group and in 4 normal subjects (20%). The rate of Ki-67 positivity was significantly higher in lichen planus and SCC groups than in normal subjects ($p<0.001$). P53 was positive in 26 patients (86.7%) in lichen planus group, in 20 patients (100%) in SCC group and in 8 normal subjects (40%). The rate of P53 positivity was significantly higher in lichen planus and SCC groups than in normal subjects ($p<0.001$). In conclusion, this study confirmed that lichen planus could be regarded as a potentially premalignant condition.

Key words: Oral lichen planus, Ki-67, P53, squamous cell carcinoma, premalignant condition

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INTRODUCTION

Lichen planus is a chronic inflammatory disease of the oral cavity in 1-5% of general population (Mansourian *et al*., 2008). The disease represents as a set of lesions including white involvements (striation, papule, plaque), erythema, erosions and blisters mainly on the mucosa, gingival structures and tongue (Roopashree *et al*., 2010). Although it is believed that a T-cell mediated autoimmune mechanism play a major role in pathogenesis of lichen planus, the exact underlying etiology is still under debate (Xue *et al*., 2005).

Oral lichen planus is generally considered as a benign entity. However, there are case reports of Squamous Cell Carcinoma (SCC) which are suspected to be developed from a previous mucosal lichen planus. The odds of such transformation are not determined (Acay *et al*., 2006).

P53 is a protein which acts as a tumor suppressor in human being in its normal form. The wild-type of this gene inhibits proliferation and oncogene-mediated proliferation and transformation. So, normal cells have enough time to repair any damaged DNA or lead the process to apoptosis. In the cases with mutated or absent protein, however, the damaged DNA can replicate. As a result, more mutations and chromosome rearrangement are expected which finally may end up in malignancy. The role of P53 has been investigated in a wide range of malignancies, such as breast (Gohari *et al*., 2006; Abdelmeguid *et al*., 2008; Kabbinejadian *et al*., 2008), bladder (Al-Qahtani and Aly, 2007), liver (Abdel-Hamid *et al*., 2005; Zekri *et al*., 2005), gastric (Rezaii *et al*., 2008), colorectal (Bidgoli *et al*., 2007) and blood-related (Al-Haggar *et al*., 2006) cancers. Ki-67 is a nuclear protein doublet with an estimated molecular weight of 395 kD. This protein can be detected on all phases of the cell cycle, except for G0 phase and exclusively in the nuclei of cycling cells. So, it could be predicted that this protein is a reliable marker of proliferating cells. This factor has been widely studied in various pathologies; such as in cases with benign and malignant skin lesions (Talghini *et al*., 2009), hepatotoxicity (El-Kott and Owayss, 2008), cancers in animals (Bin-Meferij, 2009), lung malignancy (Priya *et al*., 2011), tongue carcinoma (Sohrabi *et al*., 2009) and some bacterial infections (Rezaii *et al*., 2008).

The present study aimed to investigate and compare the rate of expression of Ki-67 and P53 in patients with oral lichen planus, oral SCC and normal subjects.

MATERIALS AND METHODS

Study design and patients: In this prospective cross-sectional study, patients with histopathologically proved lichen planus (n = 30) and oral SCC (n = 20), as well as nonsmoking normal volunteers (n = 20) were recruited from Imam Reza Teaching Hospital, Tabriz, Iran; from January 2011 through January 2012. This study was approved by the Ethics Committee of Tabriz University of Medical Sciences and performed in strict accordance with the Helsinki Declaration. Informed written consents were obtained from the participants.

Histopathology and immunohistochemistry: Fresh biopsy specimens of oral lichen planus and SCC lesions, as well as the buccal mucosa of normal subjects were obtained. Then they were fixed in 10% buffered formalin for a day. After dehydration, paraffin-embedded specimens were prepared and examined for expression of P53 and Ki-67 labeling index by a skilled pathologist.

For Immunohistochemical staining, 4 μm thick paraffin-embedded sections were dewaxed in an oven for 30 min at 56°C.

Hematoxylin and Eosin (H and E) stained sections were employed for confirmation of diagnosis.

The samples were washed in Phosphate Buffered Saline (PBS).

All the samples were pretreated with 0.5% H_2O_2 in 70% methanol for 30 min in order to have the endogenous peroxidase blocked.

To block endogenous biotin, slides were treated with an avidin-biotin block provided by the manufacturer (DAKO).

After antigen retrieval following the manufacturer's instructions, the samples were incubated with the monoclonal antibodies against Ki-67 (MIB-1, DAKO A/S, Glostrup, Denmark; monoclonal mouse; dilution 1 : 100) and P53 (Do-7, DAKO A/S, Glostrup, Denmark; monoclonal mouse; dilution 1:100) separately for 30 min at room temperature.

Tissues were then incubated with avidin–biotin-peroxidase complex (ABC, Vector Laboratories Inc, Burlington, USA). The slides were treated with diaminobenzidine (DAB) chromagen substrate according to the manufacturer's instructions and counterstained with hematoxylin.

For negative controls, the same procedure was carried out with normal serum instead of each antibody.

For positive control, a breast cancer tissue sample (for P53) and a standardized positive control block from an intraoral carcinoma for Ki-67 expression (for Ki-67) were employed.

All the prepared slides were examined by a light microscope (Siemens, Munich, Germany) at X10-40 magnification (Fig. 1).

Positive results for Ki-67 were reported when the cells presented nuclear brown-colored staining pattern. If

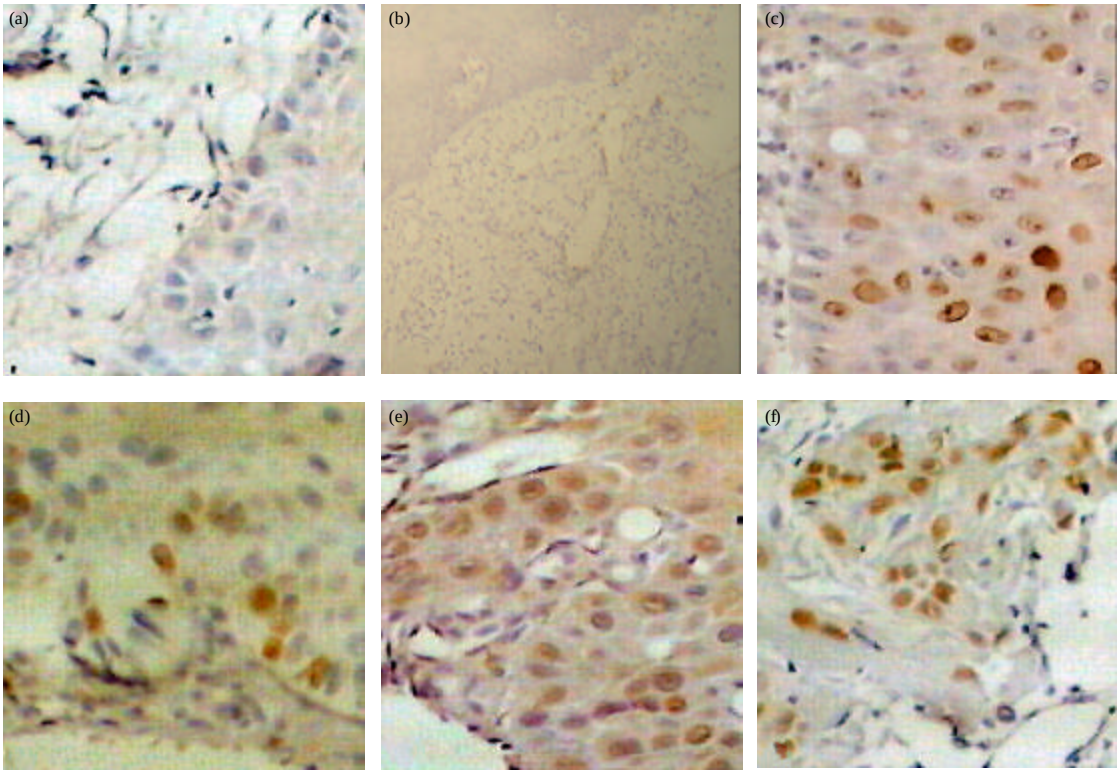

Fig. 1(a-f): Status of Ki-67 and P53 expression in various histopathologic specimens: Expression of Ki-67 in; (a) Normal tissue, (c) Lichen planus and (e) Oral SCC, Expression of P53 in, (b) Normal tissue, (d) Lichen planus and (f) Oral SCC, Hematoxylin and eosin staining, magnification: 10X (for b) and 40X (for the others)

>5% of cells within a grid area showed immunopositivity, that specimen was considered positive for expression of P53 (Taniguchi *et al*., 2002).

Statistical analysis: All variables including patients' demographics (age and gender) and the rate of Ki-67 and P53 expression were reported.

These variables were shown as Mean±SD or number (%). The SPSS software for Windows (ver.15) was used for analysis. The One-way ANOVA test or the contingency tables (chi-square test or Fisher's exact test where appropriate) were employed for analyzing the data. The $p \leq 0.05$ was considered statistically significant.

RESULTS

Thirty patients with oral lichen planus (LP group), including 10 males (33%) and 20 females (66.7%) with a mean age of 66.37±13.28 (range: 38-82) years were compared with 20 patients with oral SCC (SCC group), including 10 males (50%) and 10 females (50%) with a mean age of 64.00±11.08 (range: 57-86) years and 20 normal subjects, including 10 males (50%) and 10 females (50%) with a mean age of 67.45±10.71 (range: 48-85) years (Table 1).

Table 1: Demographic data of the studied patients and normal subjects

Variable	Lichen planus (n = 30)	Squamous cell carcinoma (n = 20)	Normal (n = 20)	p-value
Age (year)	66.37±13.28	64.00±11.08	67.45±10.71	0.69
Gender				
Male	10 (33)	10 (50)	10 (50)	0.38
Female	20 (66.7)	10 (50)	10 (50)	

Data presented as Mean±SD or number (%)

The three groups were comparable in terms of age (One-way ANOVA, p = 0.69) and gender (chi-square test, p = 0.38).

Ki-67 was expressed in 26 patients in LP group (86.7%), 20 patients in SCC group (100%) and 4 normal subjects (20%) (Fig. 2).

The rate of Ki-67 positivity was significantly higher in LP and SCC group than in normal subjects (chi-square test, $p<0.001$ for the both). It was comparable between LP and SCC groups (Fisher's exact test, $p = 0.14$).

P53 was positive in 26 patients in LP group (86.7%), in 20 patients in SCC group (100%) and in 8 normal subjects (40%) (Fig. 3).

The rate of P53 positivity was significantly higher in LP and SCC groups than in normal subjects (chi-square test, $p<0.001$ and $p = 0.001$, respectively). It was comparable between LP and SCC groups (Fisher's exact test, $p = 0.14$).

Association of Ki-67 and P53 expression in each group is summarized in Table 2. Accordingly, 24 cases with lichen planus were simultaneously positive for the both markers. Similar condition was present in 4 cases in the group with normal tissue specimens. Finally, all the cases in SCC group were positive for Ki-67 and P53.

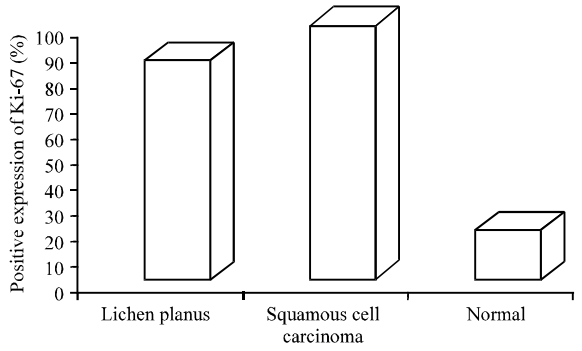

Fig. 2: Percentage of cases with Ki-67 expression in the three studied groups

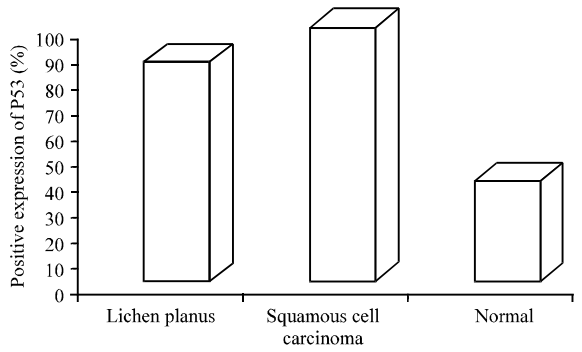

Fig. 3: Percentage of cases with P53 expression in the three studied groups

Table 2: Association of Ki-67 and P53 expression in the three studied groups

		P53	
Group	Ki-67	Negative	Positive
Lichen planus	Negative	2	2
	Positive	2	24
Normal	Negative	12	4
	Positive	0	4
Squamous cell carcinoma	Negative	0	0
	Positive	0	20

DISCUSSION

It is almost a long time that there is an ongoing debate on the nature of oral lichen planus. Despite a number of studies in the literature in this regard, there is not yet a consensus.

To depict this heterogeneity, for example while Van der Meij *et al.* (2003) found no potential risk of transformation toward SCC in their cases with oral lichen planus, Acay *et al.* (2006) concluded that oral lichen planus could be regarded as a premalignant condition.

In the present study, we examined the rate of P53 and Ki-67 expression in cases with oral lichen planus and oral SCC and recruited normal subjects as controls. Based on the immunohistochemical evaluations, both P53 and Ki-67 labeling indices were significantly more prevalent in specimens with lichen planus (86.7% for both) and SCC (100% for both) than in normal samples (40 and 20% for P53 and Ki-67, respectively). These findings indicate that there might be a potential tendency for malignancy in oral lichen planus.

In previous reports, the rate of P53 positivity varies between 40 to 70% in cases with oral lichen planus. The corresponding rates for expression of Ki-67 are between 96 to 100% (Gonzalez-Moles *et al.*, 2006; Mattila *et al.*, 2007; Sousa *et al.*, 2009). Present results are also similar to these reports.

In line with our findings, the rate of Ki-67 and P53 positivity was significantly higher in patients with lichen planus than in normal counterparts in another series by Taniguchi *et al.* (2002) , drawing a conclusion that lichen planus might be considered a premalignant disease.

Agha-Hosseini *et al.* (2009) showed that the expression of both P53 and Ki-16 markers was significantly higher in 44 specimens of oral lichen planus in comparison with 30 controls. They also concluded that oral lichen planus should be regarded as a premalignant condition.

De Sousa *et al.* (2009) also reached a similar conclusion by comparing the rate of P53 positivity between 24 cases with oral lichen planus and an equal number of patients with oral SCC. Although the results of this study are also in conformity with ours, lack of a control group is a major limitation.

This limitation was settled by Safadi *et al.* (2010), however, small sample size is another limitation in this study on 18 samples of oral lichen planus, 10 oral SCC

and 10 normal oral specimens. P53 was expressed in 40.3% of the cases with oral lichen planus, 49.9% of the cases with SCC and 15.1% of the cases with normal epithelium. They concluded that the rate of P53 expression was not significantly different between the lichen planus and SCC cases; but it was significantly higher than in normal counterparts.

It is previously shown that by 2% of all cases with oral lichen planus will lead to SCC in 5 years (Hietanen *et al*., 1999).

So, suggesting a noninvasive screening method to determine high risk cases would be of clinical significance. Both Ki-67 and P53 have been proved to be good indicators of malignancy (Gohari *et al*., 2006; Talghini *et al*., 2009).

Summarizing the results of the mentioned studies, as well as the findings of the current investigation put an emphasis on first, the premalignant status of oral lichen planus; and second, the usefulness of Ki-67 and P53 biomarkers as predictors of poor prognosis. However, the later may be examined in further long-term longitudinal studies.

We also found a strong coexistence between P53 and Ki-67 expression in oral lichen planus. This is reported in another study, too (Piattelli *et al*., 2002). This finding again further consolidates prognostic value of these two markers in patients with oral lichen planus.

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