Evaluation comparative P53 expression in Lichen Planus samples and the margins around the lesion

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ABSTRACT
Lichen planus (LP) is a chronic mucocutaneous lesion that is relatively common in the oral mucosa. Because of this lesion recurrence is common and it is considered as a premalignant lesion for this reason, the study and evaluation of molecular changes Marker p53 in lichen planus lesions and safe margins around the lesion was performed. This study is a descriptive - analytic. Study population, 60 paraffin blocks of patients with oral lichen planus in Tabriz Dental School. Immunostaining of p53 performed for sections that Obtained from paraffin blocks according to the manufacturer's instructions. Percentage of stained cells in the basal and Suprabasal layer, grading based on the degree of staining. Less than 10% (-), 11-25% (+), 26-50% (++) , 51-75 % (+++). The Data obtained from the study analyzed by descriptive Statistical methods (frequency - percent) Wilcoxon and spearman statistical methods – by using the SPSS16 software. The results of test showed that levels of P53 expression are not equal between lichen planus and safe margin tissue. 96% of expressed p53 is in lichen planus affected tissue and 26% is in the safe margins around the lesion(p-value<0.05). Expression of p53 exists in both lichen planus and safe margin around the lesion. The reason of lesions common recurrence may be remain margins that have normal appearance lichen planus. So removing some of the normal tissue surrounding is recommended for patients with lichen planus.

Introduction
Lichen Planus (LP) is a chronic and relatively common disease of the skin or mucous membranes and mostly involves oral mucosa. The majority of patients were
middle-aged women and a few were children. Although the causes are not known, the existing evidence suggests that, Lichen planus is a disease of the skin or mucous membranes that involves the immune system (Porter et al., 1997a). Hence, patients are recommended to use corticosteroids to reduce the annoying symptoms of the disease. The clinical and histological symptoms of lichen planus are the major characteristic symptoms of the disease but are not considered to be pathognomonic (Sauage et al., 2002). Description of different clinical manifestations of oral lichen planus (OLP) depends on the subepithelial inflammation (Boorghani et al., 2010).

The possibility of malignant lichen planus, especially erosive lichen planus, has been studied for a long time. The majority of reported malignancy transformations are erythroplakia dysplastic cases in which secondary lichenoid inflammatory infiltrate occurs. In such cases, conditions are similar to the lichen planus condition and the range of annual malignancy transformations of oral lichen planus varies between 0.4 and 0.174% (Boorghani et al., 2010). In oral lichen planus, tongue is involved in malignancy transformations more than other organs (Eisen et al., 2005). The majority of cases are also patients that suffer either from erosive lichen planus or a type of erosive lichen planus known as the plaque-type. The reoccurrence of this lesion is common.

Therefore, patients are recommended to use low-level steroids to eliminate their symptoms (Boorghani et al., 2010). Considering the poor prognosis of this disease, which results from the repetitive recurrences of this disease, the treatment of these patients leads to failure. Hence, photodynamic therapy, CO2 laser treatment, and cryosurgery are the treatment methods used for such patients. Excision surgery methods are also recommended for different types of plaque type lichen planus. Considering the atrophic nature of the epithelium of such lesions in erosive and atrophic cases and due to the considerable inflammations caused by these lesions, surgery is not an option because surgical changes stimulate the lesion and lead to the formation of more lesions in the region. The additional lesions will also add to the pain of the patient. Hence, surgery is not the first treatment option. Researchers also believe that such patients shall undergo periodic follow ups (ACTA, 2010). From the histological point of view, the margins around the lesion remain healthy and free of any malignancy. Therefore, molecular and genetic analysis of healthy margins is considered an important factor in preventing the failure of treatment. These analyses help identify patients at higher risks of recurrence.

P53 is a tumor suppressor gene used to prevent improper cellular proliferation and to keep genome integration following the application of genotoxic stresses. It is also used to adjust apoptosis resulting from the damages caused to DNA by mutation factors (Amaral et al., 2010). Hence, using the P53 marker it is possible to identify the products of this mutation which are mutated proteins. Numerous studies have reported on the existence of a relationship between P53 and malignancy transformations (Li et al., 2001). Research results suggest that mutation of the tumor suppressor gene P53 is the most common molecular defect found in human malignancies such as Squamous cell carcinoma (Seyedmajidi et al., 2011). The P53 market is observed in lichen planus lesions more than normal oral mucous. Results of the studies by Fakhrjou and Toutounchi (2012), Safadi et al. (2010) and Schifter et al. (1998) also confirmed the high outbreak of the P53
market in oral lichen planus lesions (Seyedmajidi et al., 2011). In this research it was tried to study for the first time the significant acknowledged role of the P53 market in the healthy and normal margins of lichen planus lesions. The results of this research were meant to be used to devise more improved treatment plans for preventing the recurrence and increase the prognosis of such patients.

**Materials and Methods**

In a descriptive-analytical study that performed in pathology unit of the dentistry department of Tabriz University of Medical Sciences on patients with lichen planus, P53 expression in lichen samples and the margins around the lesion evaluated.

The present research was a descriptive-analytical study. The study population included the paraffin blocks of patients with oral lichen planus, who had visited the pathology unit of the dentistry department of Tabriz University of Medical Sciences. The results of the studies by Li et al. (2001) were used to determine the sample size. Assuming an alpha coefficient of 0.05, a potential of 80% and 52% was assumed for the erosive group and a potential of 18% was assumed for the non-erosive group. Based on these assumptions, the sample size was estimated to be 60 (Porter et al., 1997b).

A total of 60 samples were collected from the cases available in the pathology sector of the dentistry department. Next, 3-micron cuts of the blocks were obtained and stained with H&E. The outputs were later examined by 2 pathologists in terms of previous outbreaks of lichen planus. In the end, only samples with health surrounding tissues were selected. After selecting the required lams, 4-micron cuts were obtained from the blocks. The lams were exposed to a temperature of 37 degrees for 16 hours and later to a temperature of 60 degrees for 1 to 2 hours to be fully dried and for the tissue to fully stick to the lam. Next, the lams were soaked two times in gazlene, absolute ethanol, 96% ethanol and water for 5 minutes separately. Afterwards, the samples were put in citrate buffer and were transferred to microwave. Next, lams were put in a dark room and the surfaces of lams were covered with P53 antibody. The samples were afterwards put in a 37°C temperature for 30 minutes. In the next step, the lams were put in TBS buffer for 5 minutes and were washed with water. Next, they were put in 96% ethanol, absolute ethanol and gazlene for 5 minutes. The lams were covered with cover slip and the prepared lams were examined by a pathologist with optical microscope with a magnification of 40x. The activity of P53 in stratum basal in one filed is shown in the following classification (8): less than 0-10% (-); 11%-25% (+); 26%-50% (++); 51%-75% (+++).

In order to study the relationship between P53 in the patients’ tissue and normal tissue the Spearman test was used. The data was later analyzed using SPSS version 16. Considering the non-parametric nature of data the Wilcoxon test was used to draw a comparison between the outbreak of P53 in the tissue of patients with lichen planus and the tissue surrounding the lesion. The significance level of the test was also 0.05.

**Result and Discussion**

The present study was a descriptive-analytical conducted on 60 samples. The mean age of samples was 35.7 ± 16 years and the samples aged between 15 to 78 years. Moreover, 42.9% of the patients under study were female and 57.1% were male. Results of the statistical analysis also revealed that there was no significant
difference between the expression of P53 expression a tissue with lichen planus and a normal tissue around the lesion (P=0.313) (Table 1, 2). Results also indicated that 96% of the outbreak of P53 was in tissues with lichen planus and 26% of the outbreak of P53 was in normal tissues around lesions. These results indicated that the outbreak of P53 in tissues with lichen planus is not equal to normal tissues around lesions. The outbreak of P53 in tissues with lichen planus was also higher than the outbreak of P53 in normal tissues around lesions (P<0.05).

This study demonstrated that P53 expression in tissue affected with lichen planus was more than its emergence in normal tissue around the lesion, and it had also been expressed in normal tissue around the lesion. Lichen planus is a chronic skin and mucous membrane disease prone to a potential for malignant transformation, although there is controversy over the risk rate of the malignant transformation (Boorghani et al., 2010). Schifter et al. (1998) demonstrates excessive emergence of P53 in oral lichen planus, which may be a physiological response to excessive cellular proliferation. Therefore, it decreases the value of malignancy related to oral lichen planus (Safadi et al., 2010).

The results of Schifter et al. (1998) and Safadi et al. (2010) demonstrated that the emergence value of P53 and P21 in oral lichen planus was more than that in normal mucous membrane, which is similar to the results obtained from this study (Seyedmajidi, 2011; Safadi et al., 2010; Porter et al., 1997b).

In Acay and colleagues study, P53 demonstrated an obvious difference with a higher mean in lichen planus as compared to oral lichenoid lesions, which is in accordance with the results of this study (Acay et al., 2006). In Garciar and colleagues study, the P53 staining pattern in lichen planus implies excessive wild-type protein expression (Garcia de Sousa et al., 2009).

In Fakhrju and colleagues study, P53 expression has been examined in oral lichen planus and Squamous cell carcinoma and normal tissue, and expression value of P53 in lichen planus and Squamous cell carcinoma was more than that in normal tissue, which is in accordance with the results of our study (Fakhrjou and Toutounchi, 2012).

Aghahoseyni and colleagues study performed a histochemistry analysis of P53 and Ki-67 proteins in oral lichen planus and normal oral mucous membrane. The mean expression value of P53 in patients with oral lichen planus was higher than that in the control group, which is similar to the results of our study (Agha-Hosseini et al., 2009). These results demonstrate that tissue affected with lichen planus has the potential for conversion into malignancy, and lichen planus should still be considered as a premalignant lesion.

Hietanen et al have stated that about 2% of all oral lichen planus cases convert into squamous cell carcinoma within 5 years. Therefore, they suggested that a non-invasive screening method capable of identifying high-risk cases will have high clinical value (Heinten et al., 1999).

In studies conducted by Junior using the CO2-Laser method on lichen planus patients, it was stated that no recrudescence of the lesion had been reported for a year (de Magalhaes-Junior et al., 2011). Moreover, research performed by Setterfield and colleagues, suggests the effectiveness of the method of surgery on lichen planus patients (Setterfield et al., 2000).
Table 1 P53 expression rate in Lichen Planus and normal samples

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<th>P53 expression in lichen samples</th>
<th>P53 expression in normal samples</th>
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<td>&lt;10 %(-)</td>
<td>3(%4)</td>
</tr>
<tr>
<td>11-25 % (+)</td>
<td>19(%32)</td>
</tr>
<tr>
<td>26-50 % (+++)</td>
<td>36(%3)</td>
</tr>
<tr>
<td>51-75 % (+++)</td>
<td>3(%4)</td>
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Table 2 Mean ± Std Deviation and Median of P53 expression rate in Lichen Planus and normal samples

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<th>Mean ± Std Deviation</th>
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<tr>
<td>P53 expression in Lichen Planus samples</td>
<td>32 ± 13.86</td>
<td>30</td>
</tr>
<tr>
<td>P53 expression in normal samples</td>
<td>9.57 ± 7.97</td>
<td>10</td>
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These investigations demonstrate that besides the fact that the lesion has a potential for malignancy as well as frequent recrudescence, using aggressive surgery methods including lesion removal together with normal margin around the lesion, treatment of these patients will lead to better results. Furthermore, using systemic and local medicinal methods has side effects, while using laser has high effectiveness factor without side effects (Mahdavi et al., 2013).

Considering the results of the present study, it appears that lesion removal using the surgery or laser method together with clear margin around has lower recrudescence. It is suggested that the idea be investigated in future.

In this study, besides the examination of the lesion, normal margins around the lesion have also been examined. The results demonstrate that apparently normal margin around the lesion has displayed the P53 marker, which itself can imply high recrudescence of the lesion and appearance of malignant transformation in these patients. Therefore, considering the results of our study and other research, it is recommended that more complete treatments be performed in these patients, including removal of the entire lesion together with apparently normal and clear margin around lichen planus. Moreover, considering that no similar study has dealt with a molecular examination of clear margins around lichen planus, so far, it is recommended that further similar research be conducted on the molecular-genetic structure of margins around these lesions, so that the malignance value of lichen planus and its frequent recrudescence can be evaluated with more accuracy and validity.

In conclusion, considering the results of our study and other previous studies, P53 expression in the lesion is more than that in normal tissue around the lesion, so malignancy is probable in tissue affected with lichen planus. Moreover, since P53 had also been expressed in normal tissue around the lesion, this can cause frequent recrudescence and dysplastic transformation in patients. To prove the second item, several longer-term studies with more samples should be conducted.
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