



Salivary Transferrin Levels in Patients with Oral Lichen Planus


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Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 31 August 2018 / Accepted: 05 December 2018 / Published: 11 December 2018

Abstract

Objective: To compare salivary transferrin levels between patients with oral lichen planus (OLP) and healthy subjects. **Material and Methods:** In this descriptive, analytical, cross-sectional study, 11 patients with OLP and 22 healthy subjects were selected after matching in terms of age and gender. OLP was confirmed by two oral medicine specialists based on clinical and histopathological criteria. Salivary samples were collected by spitting. The patients were asked to collect their saliva in their oral cavity and then evacuate it into sterilized Falcon tubes. The procedure was repeated every 60 seconds for 5-15 minutes. A total of 5 mL of saliva was collected using this method. The samples were collected from 8 to 9 in the morning in a fasting state to avoid circadian changes. The collected salivary samples were immediately placed next to ice and transferred to the laboratory to be centrifuged at 4°C at 800 g to isolate squamous cells and cellular debris. Then the samples were frozen at -80°C until the samples were prepared. An ELISA kit was used to determine salivary transferrin levels. Data were analyzed with descriptive statistics (means and standard deviations) and t-test for independent groups using SPSS 17. Statistical significance was set at $p < 0.05$. **Results:** The mean salivary transferrin concentrations in patients with OLP and healthy subjects were 0.9055 ± 0.28229 and 1.5932 ± 0.80041 mg/dL, respectively ($p < 0.05$). **Conclusion:** The salivary transferrin levels in patients with OLP were significantly lower than those in healthy subjects.

Keywords: Mouth Diseases; Lichen Planus, Oral; Saliva; Transferrin.

Introduction

Oral lichen planus (OLP) is a T-cell-mediated chronic mucocutaneous lesion with an unknown etiology. The condition has several forms, including reticular, popular, plaque-like, erosive, atrophic and bullous forms. The condition mostly affects middle-aged women and its prevalence rate is 5%, with an incidence of 0.5-2.3% [1,2].

OLP is a debilitating condition and is associated with symptoms and signs such as pain and burning sensation in the oral mucosa, a feeling of coarseness in the oral mucosa, a decrease in the flexibility of mucosa and a limitation in mouth opening. In addition, some researchers believe that this condition has the potential to induce malignancy, including oral squamous cell carcinoma (OSCC). Normally, a diagnosis of lichen planus is reached based on a combination of clinical and histopathological findings. However, since the lesion is usually the same color as the normal oral mucosa or is white in color in its early stages, it remains undiagnosed until its symptoms and signs appear [1,3-5]. Therefore, researchers are trying to find new techniques for the early, easy and definitive diagnosis of this condition. Saliva has drawn the attention of researchers as a readily available clinical sample, with various enzymes and molecules that have different uses for the diagnosis of various medical conditions [4,6]. Molecular markers such as DNA, RNA and other protein markers can be traced in the saliva, and these markers can be used for the early diagnosis of diseases [7,8]. Salivary biomarkers can be used for the early diagnosis of malignancies [9].

In addition, evaluation of salivary samples has other advantages, too, in that it is easy and inexpensive to collect and store salivary samples, poses minimal risks to the patient compared to other techniques and can be used as a screening test for large populations as a cost-effective and noninvasive technique [4,10].

Transferrin found in the saliva and serum is a protein of the beta globin group and is responsible for the transfer of iron between the areas of production, consumption and storage. It is one of the acute phase proteins and prevents formation of iron-mediated free radicals; therefore, it is a member of the antioxidative system of the body [4,11]. Previous studies have reported that oxidative stress reaction is one of the most important factors involved in the pathogenicity of lichen planus. In this context, the concentration of ceruloplasmin, which has an antioxidative activity, was significantly higher in the saliva and serum of patients with lichen planus [12]. In addition, the total antioxidative capacity was lower in patients with lichen planus [13]. Therefore, it is expected that transferrin levels, as an antioxidant level will be different in the serum or saliva of patients with LP compared to healthy subjects. However, a search in academic sources and databases did not bring up a study on the comparison of salivary levels of transferrin in patients with LP and healthy subjects. Only in one similar study, salivary transferrin was introduced as a biomarker for the early diagnosis of oral SCC [4].

Therefore, the aim of this study was to compare the salivary transferrin levels in patients with OLP with those in healthy subjects. It is hoped that the results of the present study would improve the diagnostic and therapeutic procedures of patients with LP, especially as a diagnostic biomarker for the early diagnosis in transition from premalignant lesions to malignant lesions.

Material and Methods

Study Design

In the present descriptive, analytical, cross-sectional study, 11 patients with different forms of OLP and 22 healthy subjects, with an age range of 20-50 years, who referred to the Department of Oral Medicine, Tabriz Faculty of Dentistry from September 2016 to September 2017, were randomly selected and evaluated. The subjects were matched in terms of age and gender. OLP diagnosis was confirmed by two oral medicine specialists based on clinical examinations and histopathological evaluations. The results of a previous study [4] were used to determine the sample size by considering $\alpha=0.05$ and a study power of 80%. A total of 11 and 22 subjects were assigned to the OLP and healthy subject groups, respectively.

The inclusion criteria were: a) Consent to be include in the study and b) Affliction with OLP. The following exclusion criteria were adopted: 1) Smoking and use of alcohol and tobacco during the previous month; 2) A history of oral surgery or trauma to the oral cavity during the previous month; 3) Use of medications interfering with the antioxidative defense system during the last three months; 4) Affliction with other systemic conditions such as diabetes, hyperthyroidism and iron deficiency anemia during the previous three months and 5) Use of vitamin supplements during the previous three months [12,13].

Data Collection

The salivary samples were collected by using the spitting method. The patients were asked to collect their saliva in their oral cavity and then evacuate it into sterilized Falcon tubes. The procedure was repeated every 60 seconds for 5-15 minutes. In order to collect unstimulated salivary samples, the subjects were asked to refrain from eating and drinking or stimulating the oral cavity mucosa for 90 minutes before collecting the salivary samples. A total of 5 mL of saliva was collected using this method. The samples were collected from 8 to 9 in the morning in a fasting state to avoid circadian changes. The collected salivary samples were immediately placed next to ice and transferred to the laboratory to be centrifuged at 4°C at 800 g to isolate squamous cells and cellular debris. Then the samples were frozen at -80°C until the samples were prepared. An ELISA kit was used to determine salivary levels of transferrin [12,13].

Data Analysis

Data were analyzed with descriptive statistics (means and standard deviations) and t-test for independent groups using IBM SPSS Statistics for Windows Software, version 17 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p<0.05$.

Ethical Aspects

This research project was approved by the Ethics Research Committee of the Tabriz Faculty of Dentistry.

Results

There were no statistically significant differences between the two groups in terms of gender ($p=0.71$) and mean age ($p=0.78$) of the participants (Table 1).

Table 1. Gender and mean age of the participants.

Group	Gender				Mean Age
	Male		Female		
	N	%	N	%	
Lichen Planus	4	36.4	7	63.6	42.64 ± 7.21
Control	10	45.5	12	54.5	41.94 ± 6.64

The mean salivary levels of transferrin were determined separately in the control and OLP groups, as presented in Table 2. The minimum, maximum and means of salivary transferrin levels were significantly higher in the control group compared to the test group.

Table 2. The minimum, maximum and mean values of salivary transferrin levels (mg/dL) in the control and test groups.

Group	N	Minimum	Maximum	Mean ± SD	p-value	95% CI
Lichen Planus	11	0.48	1.3	0.9055 ± 0.28229	0.0099	0.177063 to
Control	22	0.72	3.98	1.5932 ± 0.80041		1.198337

Discussion

Normally a diagnosis of LP is reached based on a combination of clinical and histopathological findings. However, since this lesion is usually being the same color as the oral cavity mucosa or is white in color in its early stages, it remains undetected until its symptoms and signs appear [1-3,5]. Therefore, it appears it is necessary to use new techniques for its early, easy and definitive diagnosis.

In the present study, the salivary levels of transferrin in patient with OLP were compared with those in healthy subjects. The results showed that the salivary transferrin levels in patients with OLP were significantly lower than those in healthy subjects ($p<0.05$). Consistent with the results of the present study, others authors showed that the total antioxidant capacity in patients with OLP was lower than that in healthy subjects [14]. Oxidative stress is an important factor in the induction of OLP, with a significant relationship between LP and a decrease in the total antioxidant capacity [13]. In patients with OLP there was an increase in the oxidative stress and a defect in the antioxidative system [15]. Therefore, a disturbance in the balance of oxidative stress has been shown in OLP patients, and since salivary transferrin has an antioxidative activity, a decrease in its levels in OLP patients is justifiable.

In contrast, some studies reported results different from those described here. A previous showed that superoxide dismutase (SOD) antioxidant serum levels in patients with LP were significantly higher than those of healthy subjects [16]. The discrepancy between the results of that study and those of the present study might be attributed to the evaluation of serum levels of SOD

instead of its salivary levels and the differences in the study populations between these two studies. Oxidative stress reaction is one of the most important factors in the pathogenicity of LP and the salivary and serum levels of ceruloplasmin, which has antioxidative activity, was significantly higher in patients with LP compared with healthy subjects [12]. However, in the present study, the salivary levels of transferrin in patients with LP were less than those in healthy subjects.

Given the complexity of the body's defense system, especially the antioxidative system, it is recommended that all the factors involved in the process be evaluated simultaneously, rather than evaluation of individual components of the antioxidative system such as salivary transferrin, so that the results would be more pertinent to reality. For example, in future studies, researchers might evaluate the relationship of transferrin and ceruloplasmin with OLP at the same time instead of evaluation of only the relationship of transferrin and OLP. In addition, evaluation of the specific relationship of different forms of LP with antioxidative factors might help improve the results. It is obvious that increasing the sample size can decrease biases in the results.

Saliva can be used as a diagnostic fluid in all the age groups because collection of saliva is associated with fewer compliance problems compared with collecting blood samples [4,10]. However, salivary levels of certain markers do not always reflect the serum levels of these markers. Detection of serum components, which are not part of the normal salivary constituents in the saliva, depends on the physicochemical characteristics of these molecules. Composition of the saliva can be affected by the method used for collecting samples and the degree of stimulation of salivary flow. In addition, proteolytic enzymes of the saliva might affect the stability of certain diagnostic markers [12,17,18]. Therefore, further studies are necessary to more accurately evaluate salivary markers for the diagnosis and treatment of patients.

Conclusion

The salivary levels of transferrin in patients with OLP were significantly less than those in healthy subjects.

Financial Support: Faculty of Dentistry, Tabriz University of Medical Sciences.

Conflict of Interest: The authors declare no conflicts of interest.

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