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Original Article

Zinc Finger Protein 510 Levels in the Saliva of Patients with Oral Lichen Planus Compared with Healthy Controls

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ABSTRACT

Background: The aim of this study was to evaluate the concentration of zinc finger protein 510 (ZNF510) in the saliva of patients with oral lichen planus and healthy individuals in 2019.

Methods: This cross-sectional analytical study was performed on 24 patients with oral lichen planus and 25 healthy individuals referred to the School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran, between June and October 2019. In the case group, the severity of lesions was determined according to the Thongprasom index. Unstimulated saliva was collected from the subjects and the samples were examined for the presence of the ZNF510 protein using ELISA method. The data were statistically analyzed through SPSS 23. For data analysis, Kolmogorov-Smirnov test, the independent t-test, the independent samples Kruskal-Wallis test, and one-way analysis of variance were used.

Results: This study included 32 females (65.3%), and 17 males (34.7%). The subjects' age range was between 23-70 years and the mean age of them was 46.26 ± 10.90 years. The mean ZNF510 (ppm) in the case group was 86.12 ± 34.88, while in the control group, it was 46.43 ± 23.32. The two groups were significantly different in terms of the mean ZNF510 ($P < 0.001$). In patients with non-keratotic lichen planus, the mean ZNF510 was significantly higher than that in those with keratotic lesions ($P = 0.028$). Moreover, in patients with oral lichen planus, the severity of lesions according to the Thongprasom index was significantly and directly related to the ZNF510 concentration ($P = 0.002$).

Conclusion: The concentration of ZNF510 protein in the saliva can be a good indicator for assessing the severity of oral lichen planus lesions and its diagnosis. However, its clinical application is possible only if extensive prospective studies are performed.

Keywords: Zinc finger protein, Oral lichen planus, Saliva

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Introduction

Oral lichen planus (OLP) is a chronic immunological, mucocutaneous disorder, in which the oral cavity is often involved. It usually occurs in people between the ages of 30 and 60 years, mainly women (1-3). Its prevalence varies in different populations between 0.5 to 2.2% (3, 4). The lesions appear in various types in terms of clinical manifestations, including reticular, papule, plaque, ulcerative, atrophic erosive, and bullous types (2, 5, 6). One of the major problems with OLP is the potential to malignantly develop into oral squamous cell carcinoma (OSCC). Although OLP has been recognized by the World Health Organization (WHO) primarily as a precancerous condition, reports about its possible development toward malignancy are inconsistent (2).

Diagnosing and treating OLP in the early stages prior to invasion offers the best prognosis. New tumor markers are essential for the detection of precancerous oral lesions, especially in those at high risk. Saliva is a good candidate for biomarker identification. Compared to biopsy, the use of saliva to screen for precancerous lesions is a simpler and less invasive method and is more easily tolerated by patients (7, 8).

Zinc finger protein 510 (ZNF510) is a protein that is encoded by the ZNF510 gene in humans. It belongs to a family of about 30 amino acids and zinc ions that are involved in extraordinarily diverse functions including control of cell growth, proliferation, differentiation and apoptosis (9, 10). Some types of zinc finger proteins are also involved in a wide range of biological processes related to immune responses such as cytokine production, immune cell activation, and immune homeostasis, as well as in regulating cell differentiation and cancer cell growth (11).

ZNF510 has been observed in the saliva of patients with OSCC (9). Jou *et al.* study revealed that ZNF510 levels are significantly increased in the saliva of OSCC patients, and strongly associated with OSCC stages. They concluded that salivary levels of ZNF510 peptide serves as an early-stage biomarker for OSCC (9).

To date, no study has examined the ZNF510 protein in the saliva of patients with OLP as a premalignant lesion. OLP is a chronic immunological, mucocutaneous disease and is possibly associated with OSCC development (12, 13).

Therefore the present study aimed to evaluate the concentration of ZNF510, as a new marker, in the saliva of patients with oral lichen planus and healthy subjects.

Materials and Methods

This cross-sectional analytical study was conducted on patients with OLP and healthy individuals who were referred to the School of Dentistry at Mashhad University of Medical Sciences, Iran, in 2019. The study protocol was approved by the ethics committee of the research council at Mashhad University of Medical Sciences (Ethical approval code: IR.MUMS.DENTISTRY.REC.1397.119.).

Inclusion criteria were patients with oral lichen planus confirmed by clinical and histopathological examination and were consent to participate, patients over the age of 18 years and those who had not received any systemic or topical lichen planus medication or vitamin supplements. Exclusion criteria were patients with systemic diseases associated with immune disorders such as a history of diabetes, chemotherapy and radiation therapy, pregnancy or breast feeding (9), the presence of oral mucosal lesions, drug-induced and contact lichenoid reactions, and Graft versus host disease (GVHD).

A number of 24 patients with OLP and 25 healthy individuals were selected and evaluated. They were initially given a full explanation of the study and agreed to participate by signing a written consent. The participants were then divided into the case and control groups and a checklist including age, gender, medical and drug use history, and for patients, the type of lesion, its location and severity was prepared. The type of lichen planus (keratotic, non-keratotic) was confirmed by clinical examination and the severity of the lesions was classified according to the Thongprasome index (14) as follows: 0 : no lesion/ normal mucosa, 1: mild white striae/no erythematous area, 2: white striae with atrophic area less than 1cm², 3: white striae with atrophic area more than 1cm², 4: white striae with ulcerative area less than 1cm², 5: white striae with ulcerative area more than 1cm². The control group consisted of healthy individuals with no oral lesions.

Next, the saliva samples were taken from the participants. Since stimulated saliva has a low concentration of biomarkers which makes detection difficult, unstimulated saliva was used for the sampling (15). Participants were advised

to abstain from smoking and drinking for 24 hours before collecting saliva. On the day of sampling, patients were asked not to smoke, eat, drink, or brush their teeth for about 1.5 hours before collecting saliva. The unstimulated saliva was collected using the spitting method (16). The participants were asked to collect their saliva into a sterile plastic tube (flacon). This was done about every minute and for 5-15 minutes. Approximately 5 ml of saliva was obtained by this method. The saliva was collected from the participants between 9 a.m. and 12 a.m as they were comfortably in sitting position, and bent slightly forward.

The saliva samples were transferred in an ice flask to the central laboratory of Mashhad University of Medical Sciences and stored in the refrigerator at −20°C for up to 3 days. The samples were then thawed at room temperature and were immediately centrifuged for 15 minutes (3000g at 25°C) to separate squamous cell carcinoma, debris and mucus. The supernatant from the samples was collected and poured into 1.5 microtubes and then stored in a refrigerator at −80°C. The ZNF510 protein levels (ppm) were then determined using the ZNF510 ELISA kit (ZellBioGmbH, Germany) and the ELISA method. For this purpose, each sample was examined twice by an experienced technician who was unaware about the participants' groups.

In summary, the procedure comprised 1) sample preparation and standardization, 2) adding 40 µl of the sample, 10 µl of ZNF510, 50 µl of the standard solution, and 50 µl streptavidin-HRP, and letting them react for 60 minutes at 37°C, 3) washing the plate 5 times with 300 µl of the diluted buffer, 4) adding 50 µl of the chromogen A solution and 50 µl of the B solution, and incubating for 10 minutes at 37°C, 5) adding 50 µl of the stop solution, 6) reading the optical density within 10 minutes at a wave length of 450 nm, and 7) performing calculations and preparing reports.

Statistical analysis

SPSS 23 software was used to analyze the data. The mean, standard deviation and standard error of the ZNF510 concentration in the saliva samples from the patients with OLP and healthy subjects were measured and reported. The data were analyzed using Kolmogorov-Smirnov test, the independent t-test, the independent samples Kruskal-Wallis test, and one-way analysis of variance. The significance level was set at 0.05.

Results

A total of 49 people who met the inclusion criteria participated in this study, including 32 women (65.3%) and 17 men (34.7%). They were between 23 and 70 years old with a mean age of 46.26 ± 10.90 years. The subjects were examined in the two groups, namely the case group (patients with lichen planus) and the control group (healthy individuals). The two groups did not differ significantly in terms of the mean age and gender (P = 0.304 and P = 0.084 respectively).The mean ZNF510 concentration in the case group was 86.12 ± 34.88 ppm and it was 46.35 ± 32.32 ppm in the control group. The groups differed significantly in terms of the mean ZNF510 concentration (P <0.001, Table 1).

Table1. Comparison of the mean ZNF510 in the study groups

Group	Mean	Std. deviation	Min.	Max.	Independent t-test
Patients (N=24)	86.16	34.88	25.31	168.54	T = 4.71 P < 0.001
Healthy (N=25)	46.35	32.32	2.40	46.91	

Moreover, the mean ZNF510 concentration in the case group was 88.61 ± 34.61 ppm in women and 76.67 ± 38.23 ppm in men, which was not statistically significant (P = 0.508). In the control group, the mean ZNF510 was 89.37±45.24 ppm in women and 95.08 ± 23.46 ppm in men, which was also not statistically significant (P = 0.913). As shown in Figure 1, there was a weak non-significant direct relationship between age and the concentration of ZNF510 protein in both the case and control groups.

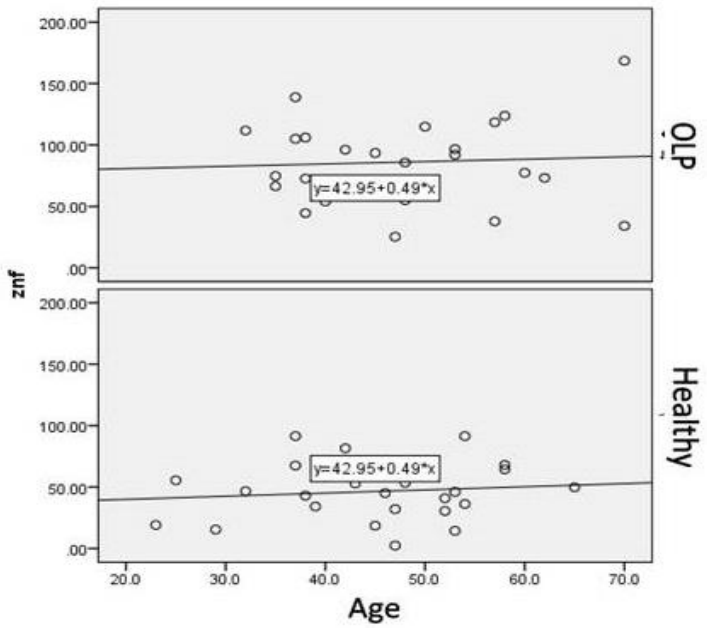

Figure 1. Distribution of ZNF510 by age and study groups

In patients with keratotic OLP, the mean ZNF510 was 62.22± 20.34 ppm, while in patients with non-keratotic OLP, it was 95.96 ± 35.22 ppm, which was statistically significant (P=0.028, Table 2). Nevertheless, the Thongprasom scores were directly related to the ZNF510 protein concentration in all patients. There was also a significant relationship between the two variables in all patients (P=0.028 and P = 0.002, Table 3).

Table 2. Comparison of the mean ZNF510 in patients with keratotic and non-keratotic OLP

Group	Lesion	Mean	Std. deviation	Min.	Max.	Independent t-test
Patients	Keratotic (N=7)	62.22	20.34	37.81	91.98	T = 2.36 P = 0.028
Healthy	non-keratotic (N=7)	95.96	35.22	52.31	168.54	

Table 3. Correlation between Thongprasom scores and ZNF510 concentrations by keratotic and non-keratotic patients

	Kertotic (N = 7)	Non-keratotic (N = 17)	Total (N = 24)
Spearman correlation coefficient	0.577	0.533	0.591
p-value	0.175	0.028	0.002

Discussion

The results indicated that the mean salivary ZNF510 protein concentration in patients with OLP was significantly higher than that in the healthy subjects. The mean ZNF510 was significantly higher in patients with non-keratotic OLP compared to patients with keratotic OLP. In addition, the severity of OLP lesions according to Thongprasom index was directly and significantly related to ZNF510 concentrations in all patients.

OLP is a chronic immunological, mucocutaneous disease caused by chronic inflammation due to epithelial cell apoptosis mediated by autocytotic T lymphocytes. According to the WHO, OLP is considered a potentially malignant oral disorder (OPMD) (17-20). According to a systematic review, several long-term prospective studies have shown that the chance of its development into malignancy over a period of 5 years is 1% (21). Given the importance of early diagnosis in treatment, it is essential to use sensitive and reliable biochemical tests for early diagnosis of the disease. Saliva contains a variety of proteins, peptides, nucleic acids, electrolytes, and hormones that originate from several regional and systemic sources (22, 23). The use of saliva is a non-invasive method compared to oral biopsy (22).

Several biomarkers in saliva have been reported to identify OLP. The levels of reactive protein C, immunoglobulin A (IgA), cortisol, matrix metalloproteinase 8, CTX I, CD14 and toll-like receptor-2, defensin-1, urinary prokallikrein and IL-6, OHdG-8 and MDA are

higher in the saliva of patients with OLP. However, the PLUNC, uric acid, TAC and GPx levels in the saliva of patients with OLP are lower compared to healthy individuals (7, 23-29).

Talungchit *et al.* reported, using proteomic method, that the expression of fibrinogen fragment D and complement component C3c was higher in the saliva of patients with OLP compared to healthy subjects, while the expression of cystatin SA was lower. Complement C3c, fibrinogen fragment D and cystatin SA can be used as salivary biomarkers in OLP screening and diagnosis (4).

ZNF proteins are a family of about 30 amino acid residues and zinc ion containing small diagnostic DNA motifs (30, 31). The ZNF510 protein is encoded by the ZNF510 gene and is a member of the krueppel C2H2-type zinc-finger protein family.

ZNFs have a variety of molecular functions and are involved in the regulation of several cellular processes with different functions. In fact, ZNFs are involved in the regulation of transcription, protein degradation, signal transduction, DNA repair, cell migration, and many other processes (30). Some types of ZNFs also play role in a wide range of biological processes related to immune responses such as cytokine production, immune cell activation, immune homeostasis, innate antiviral immunity, regulating cell differentiation, and cancerous cell growth (11).

The aim of this study was to evaluate theZNF510 protein concentration in the saliva of patients with OLP and healthy individuals in the city of Mashhad, Iran, in 2019 using the ELISA method.

To the best of our knowledge, only one study has examined salivary ZNF510 concentrations in patients with OSCC. In Jou *et al.* study in 2011, the salivary ZNF510 concentration was the only biomarker that increased with the increase of cancer stage population from T1/T2 to T3/T4 and was significantly associated with oral cancer stage and its sensitivity and specificity in the early and advanced tumor stage diagnosis was 96%. The concentration of salivary ZNF510 protein can also predict oral cancer progression (9).

In this study, salivary ZNF510 protein concentrations in patients with OLP, as a precancerous lesion, was significantly higher than that in healthy subjects. This result can be attributed to the fact that some proteins of the ZNF family can play a role in immune responses and autoimmune diseases by affecting the production of cytokines, immune cell activation and immune homeostasis.

In order to achieve a reliable cutoff, it is recommended that future studies evaluate the salivary ZNF510 protein in OLP patients with and without dysplasia as well as different degrees of dysplasia.

Conclusion

We showed in this study that the concentration of salivary ZNF510 protein in patients with oral lichen planus was higher than that in healthy subjects, and a statistically significant relationship was observed between the ZNF510 concentration and the severity of oral lichen planus lesions according to the Thongprasom index. Therefore, the concentration of ZNF510 protein can be a good indicator to assess the severity of oral lichen planus lesions and its diagnosis. However, further studies with a larger sample size are needed in order to generalize the results, and clinical application is possible if extensive prospective studies are performed.

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