

Original Article

Salivary levels of interleukin-8 in oral lichen planus and diabetic patients: A biochemical study

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ABSTRACT

Background: This study aimed to assess the salivary levels of interleukin-8 (IL-8) in oral lichen planus (OLP) and diabetes mellitus (DM) patients, and OLP + DM patients in comparison with healthy individuals.

Materials and Methods: This descriptive cross-sectional study was conducted on 75 patients (30 with OLP, 5 with both OLP and DM, 20 with DM and 20 healthy controls). The salivary levels of IL-8 and fasting blood sugar and 2-h postprandial blood glucose levels were measured in all the subjects. Data were analyzed with one-way ANOVA and *post hoc* least significant difference tests.

Results: The mean salivary level of IL-8 was the highest in OLP + DM patients, followed by DM, OLP and control groups, respectively. Pair-wise comparisons of the groups revealed significant differences in the salivary levels of IL-8 between OLP and control, DM and control, also OLP + DM patients and control ($P < 0.05$).

Conclusion: The increasing salivary level of IL-8 in the control, OLP, DM, OLP + DM groups, respectively, indicates the role of this inflammatory cytokine in the pathogenesis of OLP and diabetes.

Key Words: Diabetes mellitus, interleukin-8, lichen planus, saliva

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INTRODUCTION

The etiology of oral lichen planus (OLP) has yet to be clearly understood; however, there is a strong theory that T-cell-mediated immune responses are involved in its pathogenesis. Local and systemic release of different cytokines from blood cells and oral mucosal cells are responsible for the initiation and progression of OLP.^[1] In recent years, the primary role of immune system in the development of this condition has become more prominent. Histopathological characteristics of the disease

also confirm this theory. In general, OLP is a multifactorial condition and comprises a series of events that occur at different times during the course of disease.^[2]

Interleukin-8 (IL-8) is an important mediator of host response to injury and inflammation. Its role is to activate neutrophils, neutrophil chemotactic factor, T cells and basophils. It is produced by different cells, including monocytes/macrophages, T cells, neutrophils, endothelial cells, fibroblasts

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and keratinocytes during the inflammatory and pathological processes.^[3]

The concentration of IL-8 is insignificant in healthy tissues; however, its level rapidly reaches 10–100 times its baseline value in response to pro-inflammatory cytokines, namely tumor necrosis factor-alpha (TNF- α) and IL-1 as well as bacterial or viral products and cellular stress. In patients with OLP, keratinocytes also produce IL-1 and TNF- α .^[4] Moreover, mononuclear cells infiltrating around the OLP mucosal lesions can produce TNF- α .^[5] Keratinocytes, macrophages, T-cells, endothelial cells and fibroblasts stimulated by IL-1 and TNF- α in OLP lesions can release significant amounts of IL-8. This cytokine further enhances the infiltration of T cells, particularly cytotoxic T cells, around OLP lesions; therefore, it plays a role in the pathogenesis of OLP.^[6]

Rhodus *et al.*^[7] demonstrated that the salivary and serum levels of IL-8 were significantly higher in OLP patients compared to healthy individuals. Zhang *et al.*^[8] showed that salivary and serum levels of IL-8 and other pro-inflammatory cytokines were higher in OLP patients compared to healthy subjects and suggested that saliva can be an efficient alternative to serum for testing pro-inflammatory cytokines in OLP patients. Sun *et al.*^[6] reported that IL-8 levels were significantly higher in OLP patients compared to healthy controls and concluded that IL-8 is a more sensitive marker than IL-6 for the detection of OLP.

Noninsulin-dependent diabetes mellitus (DM) or type II diabetes is the most common form of diabetes and results from a combination of obesity, inflammation and hyperglycemia.^[9]

Zozulińska *et al.* in 1999 reported that the serum levels of IL-8 in diabetics were significantly higher than those in nondiabetics.^[10] At high glucose levels, IL-8 is produced following the adhesion of monocytes to endothelial cells in diabetic patients.^[11] Subsequently, increased IL-8 levels enhance the inflammatory reactions in DM patients. In fact, a vicious circle is created within which IL-8 and increased glucose levels mutually affect each other.

A relationship between DM and lichen planus seems possible^[12] and evidence shows that such correlation might exist.^[1,2,13,14] However, previous studies in this regard are different in terms of methodology and have yielded controversial results.^[12-14] Seyhan *et al.*^[9] reported that half of the patients with OLP have impaired glucose metabolism and ¼ suffer from DM.

Based on previous studies, IL-8 has a potential role in OLP and DM. Thus, this study aimed to measure the salivary level of IL-8 in 4 groups of OLP, DM, OLP and DM and healthy controls. Considering the possible role of IL-8 in both conditions, IL-8 levels might be higher in subjects suffering from both conditions. If this hypothesis is accepted, we may be able to take a step forward in the treatment of these patients by new modalities and host immunomodulation.

MATERIALS AND METHODS

This study was approved in the Ethics Committee of Isfahan University of Medical Sciences, School of Dentistry. This descriptive, analytical, cross-sectional study was conducted on patients with OLP, DM, and both conditions as well as healthy controls. The patients were chosen from those presenting to the Dental Clinic of School of Dentistry, Isfahan University of Medical Sciences and private dental clinics in Isfahan.

Healthy controls were selected from subjects presenting to the laboratories for regular checkups, who had no history of drug intake or systemic diseases.

Diabetic patients were selected among those referred to the Diabetes Center, suspected of having type II diabetes. These patients had fasting blood sugar (FBS) ≥ 126 mg/dL or 2-h postprandial blood glucose (2 hpp) ≥ 200 mg/dL and also exhibited the clinical symptoms of diabetes, including polyphagia, polydipsia and polyuria.^[15] These patients were not on medication and had no other systemic diseases.

Patients with symptomatic OLP were selected from those presenting to the Dental Clinic of School of Dentistry, Isfahan University of Medical Sciences and private dental clinics in Isfahan. After observing the red and white patches by an oral medicine specialist and taking a history, a biopsy was taken. Based on the pathology report, clinical diagnosis of the clinician and patient history, patients with OLP were differentiated from those with lichenoid reactions according to the World Health Organization criteria.^[16] Patients with lichenoid drug reactions, contact dermatitis, graft versus host disease and cutaneous lesions were excluded from the study.

FBS and 2 hpp blood glucose tests were performed for patients with OLP and based on the test results they were assigned to OLP or OLP plus DM groups.

This process continued until a sample size of 35 subjects was achieved.

Patients taking medications, suffering from systemic conditions other than DM and OLP, with inflammation in other parts of the body and also those with periodontal diseases were excluded from the study. Cigarette smoking, substance abuse and alcohol consumption were also among the exclusion criteria for the four groups.

However, considering the fact that DM and gingival inflammation exacerbate each other in a vicious circle,^[17] complete exclusion of DM patients with gingival inflammation was not feasible. However, we tried our best to exclude DM patients with severe gingival inflammation.

After 8 h of fasting, 3 mL of blood was obtained between 7 and 9 a.m. (to prevent circadian effects) from subjects and they were asked to have a regular (carbohydrate) breakfast in 5–10 min and come back 2 h after the breakfast for 2 hpp blood glucose test. The subjects were requested to refrain from any vigorous physical activity during this time. After obtaining FBS and before having breakfast, 3 mL of saliva was collected from each patient. Unstimulated salivary samples were obtained using the spitting technique. The blood samples obtained from the patients were centrifuged immediately after clotting at 3000 rpm for 15 min. FBS and 2 hpp blood glucose levels were measured using the separated plasma. The salivary samples were immediately stored at -20°C for later measurement of IL-8 concentration using the ELISA kit (Quantikine ELISA, R and D Systems Inc., USA and Canada).

RESULTS

Twenty type II DM patients, 20 healthy controls and 35 OLP patients were evaluated. In OLP patients, 14.28% had type II diabetes; in addition, 20% of OLP patients had impaired FBS, 100–125 mg/dL. As observed in Table 1, the mean salivary level of IL-8 was the highest in patients with both OLP and type II diabetes (417.12 ± 43.9 pg/mL), followed by DM group (382.35 ± 95.7 pg/mL),

OLP group (314.31 ± 83.8 pg/mL) and the control group (254.3 ± 77.05 pg/mL).

One-way ANOVA showed significant differences between the groups in salivary levels of IL-8 ($P = 0.001$).

Post hoc least significant difference test was then applied, revealing that significant differences existed in the salivary levels of IL-8 between the OLP and control groups ($P = 0.016$), type II DM and control groups ($P < 0.001$), control and OLP + DM groups ($P < 0.001$) and also OLP and OLP + DM groups ($P = 0.013$). Although the mean concentration of IL-8 in the saliva of OLP + DM patients was higher than that in type II diabetes group, this difference was not statistically significant ($P = 0.41$).

The mean FBS and 2 hpp blood glucose values were the highest in OLP patients with DM, followed by DM, OLP and control groups, in a descending order [Table 2].

DISCUSSION

First objective: Comparison of interleukin-8 salivary levels between the oral lichen planus patients and the control group

The etiology of OLP has yet to be fully understood. However, a strong theory suggests that T-cell-mediated immune responses (band-like infiltrates of macrophages and CD4^{+} cells in the initial phases and dominance of CD8^{+} cells in later stages) are involved in its pathogenesis. Local and systemic release of different cytokines from the oral mucosal cells and blood cells are responsible for the initiation and progression of OLP.^[1]

For years, researchers have attempted to find a suitable noninvasive method for constant monitoring of the course of lichen planus and its treatment. Many previous studies have searched for an accurate, cost-effective and noninvasive diagnostic technique for the assessment of cytokines related to this disease in blood and other diagnostic media.

The current study showed that the mean salivary level of IL-8 was significantly higher in OLP patients than the control group ($P = 0.016$).

Table 1: The mean (\pm standard deviation) changes in the salivary levels of IL-8 in the 4 study groups

IL-8 salivary concentration (pg/mL)	Control	OLP and type II DM	Type II DM	OLP
Range of changes	89-381	356-480	219.6-618.2	202.9-512
Mean \pm SD	254.3 \pm 77.05	417.12 \pm 43.9	382.35 \pm 95.7	314.31 \pm 83.8

Table 2: The mean FBS and 2 hpp blood glucose values in the 4 study groups

Group	Mean FBS (mg/dL)	Mean 2 hpp blood glucose (mg/dL)
OLP + DM	159.25±49.3	224.35±62.2
DM	152.8±26.6	210.2±44.8
OLP	99.85±14.4	109.95±36.92
Control	92.83±12.01	104.56±32.2

Rhodus *et al.*^[18] stated that salivary and serum levels of IL-8 were significantly higher in patients with lichen planus than the control group and also mentioned that IL-8 possesses diagnostic and monitoring capabilities and can help with the treatment planning process.

Zhang *et al.*^[8] reported that IL-8 level in the oral fluids of OLP patients was significantly higher than that in healthy controls and stated that evaluation of saliva samples is an efficient technique to detect high levels of cytokines related to OLP compared to the assessment of serum. They also mentioned that salivary IL-8 is a reliable biomarker for the assessment of the severity of OLP. Tavangar *et al.*^[19] showed that serum level of IL-8 in OLP patients was significantly higher than that in healthy individuals ($P = 0.002$).

Increased salivary concentrations of cytokines in OLP patients might be attributed to their increased release by the inflammatory cells or keratinocytes.^[20] On the other hand, injured oral mucosa (particularly in erosive lichen planus) can no longer act as a protective barrier and this issue may also contribute to the increase in salivary levels of cytokines.

In OLP patients, keratinocytes also produce IL-1 and TNF- α .^[6] Moreover, mononuclear cells infiltrating the tissue around OLP mucosal lesions can release TNF- α .^[5] Keratinocytes, macrophages, T cells, endothelial cells and fibroblasts stimulated by IL-1 and TNF- α in OLP lesions can release significant amounts of IL-8. This cytokine further enhances the infiltration of T cells, particularly cytotoxic T cells, at the site of OLP lesions, thus playing a role in the pathogenesis of OLP.^[6]

Second objective: Comparison of interleukin-8 salivary levels between the type II diabetic patients and the control group

In this study, the mean salivary level of IL-8 in diabetic patients was significantly higher than that in healthy controls ($P < 0.001$). Several studies have reported significantly higher serum levels of IL-8 in patients with DM than healthy controls.^[10,11] Since

factors present in the serum also enter saliva, it may be concluded that high serum levels of IL-8 can also increase its salivary concentration. Zozulińska *et al.* in 1999 reported that the serum level of IL-8 in diabetics was significantly higher than that in nondiabetics.^[10] At high glucose concentrations, IL-8 is produced following the adhesion of monocytes to endothelial cells in diabetic patients.^[11] Subsequently, increased IL-8 levels enhance the inflammatory reactions in DM patients. In fact, a vicious circle is created within which IL-8 and increased glucose levels mutually affect each other. Saliva is a complex combination of salivary gland secretions, crevicular fluid, etc;^[21] and obviously IL-8 concentration is influenced by these sources. For instance, oral inflammation increases the salivary concentration of IL-8.^[22,23]

Third objective: Occurrence of diabetes mellitus and glucose impairment in oral lichen planus patients

Previous studies have yielded controversial results in this respect; which might be attributed to differences in the methodologies and designs of studies. Atefi *et al.*^[24] in 2012 reported a prevalence rate of 20% for DM in patients with OLP. Furthermore, they reported the incidence of impaired FBS to be 17.5% (100–125 mg/dL). Ansar and Ghasemzadeh^[25] in 2012 reported a prevalence of 3.3% for DM in OLP patients in Iran. In this study, 14.2% of OLP patients had DM. Impaired FBS was seen in 20% of OLP patients. Higher prevalence of glucose intolerance in the OLP compared to the control group indicates the possible role of glucose intolerance in the pathogenesis of lichen planus.^[26]

Fourth objective: Comparison of interleukin-8 salivary levels between patients with both oral lichen planus and diabetes mellitus and other groups

In our study, salivary concentration of IL-8 in patients with OLP and DM was significantly higher than that in the control and OLP groups ($P < 0.05$). The increasing salivary and serum levels of IL-8 in the control, OLP and OLP + DM groups, respectively, indicate the role of this inflammatory cytokine in the pathogenesis of OLP and DM and the synergistic effect in patients suffering from OLP and DM.

As we have seen no study is available on salivary levels of IL-8 in four groups simultaneously. This study follows the research carried out by the same

researcher on the serum levels of IL-8 in these four groups. Tavangar *et al.* research that was carried out in the same patients showed that the serum levels of IL-8 in OLP patients and OLP + DM patients were significantly higher than those in healthy people ($P < 0.05$). Although the mean serum concentration of IL-8 in diabetic patients was more than that in the control groups, the differences were not statistically significant. In the current study, salivary levels of IL-8 in OLP patients, diabetic and OLP + DM patients were significantly higher than those in healthy individuals. It seems that salivary sampling is more convenient and noninvasive than serum sampling. The salivary sampling method can be an adjunctive or alternative method of serum sampling.

One of the limitations of this study was a lack of cooperation for taking part in this study and difficulty finding sufficient qualified OLP patients. In addition, despite the inclusion of patients without systemic or inflammatory diseases, elimination of mild internal inflammation was not possible that might have a minor effect on IL-8 level.

Future studies with larger sample sizes are required to assess and compare the salivary levels of IL-8 in different forms of OLP and investigate its effect on the clinical course of the disease.

CONCLUSION

Salivary levels of IL-8 increases in both OLP and DM as a pro-inflammatory cytokine, but its levels are significantly higher in patients with both conditions. Regulation of this inflammatory factor might lead to an improvement in clinical symptoms of OLP, especially OLP + DM.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

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