

Original Article

Serum level of Interleukin-8 in subjects with diabetes, diabetes plus oral lichen planus, and oral lichen planus: A biochemical study

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ABSTRACT

Background: Correlation between diabetes mellitus (DM) and oral lichen planus (OLP) seems probable. Since Interleukin-8 (IL-8) is an important inflammatory mediator involved in both conditions, this study aimed to measure and compare the serum level of IL-8 in DM, OLP, and DM + OLP patients in comparison with healthy individuals.

Materials and Methods: This cross sectional study was conducted on 75 patients (30 OLP, 5 OLP and type II DM, 20 type II DM, and 20 healthy controls). Serum levels of IL-8, fasting blood sugar (FBS) and 2-h postprandial blood sugar were measured in the four groups. Data were analyzed using SPSS version 20 by one-way ANOVA and post_hoc least significant difference test.

Results: Type II DM patients with OLP had the highest mean serum level of IL-8 followed by OLP, DM and control groups, respectively. Pairwise comparison of groups revealed significant differences in serum IL-8 between the control and OLP and also control and OLP+DM ($P < 0.05$) groups. No other significant differences were noted. The mean levels of FBS and 2-h postprandial blood sugar were the highest in OLP+DM patients followed by DM, OLP and control groups, respectively.

Conclusion: The ascending trend of serum level of IL-8 in the control, DM, OLP, and DM+OLP patients may indicate the role of this factor in the pathogenesis of DM and OLP. Moreover, it may play a synergistic role in patients suffering from both conditions.

Key Words: Diabetes mellitus, interleukin-8, lichen planus, oral, serum, type 2

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INTRODUCTION

Oral lichen planus (OLP) is a T-cell mediated inflammatory disease of the oral mucosa. The etiology of OLP is unknown and it occurs in 0.5% -1.9% of the population.^[1,2] Interleukin-8 (IL-8) is an important mediator of host response to injury, trauma and inflammation^[3] and plays a role in activation of neutrophils, neutrophil chemotactic factor, T-cells and basophils.^[4] Interleukin-8 is produced by different cells

such as monocytes/macrophages, T-cells, neutrophils, endothelial cells, fibroblasts and keratinocytes in inflammatory and pathological processes.^[3,5,6] Healthy tissues have insignificant amounts of IL-8 but its concentration quickly reaches 10-100 times its baseline level in response to pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) or IL-1 β , bacterial or viral products, and cellular stress.^[7]

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Keratinocytes in OLP patients can synthesize IL-1 and TNF- α .^[8,9] Moreover, mononuclear cells infiltrated in the tissues in mucosal OLP patients, as well as mononuclear cells in the peripheral blood of these patients, can produce TNF- α .^[8-10] Furthermore, following an increase in releasing both locally and systemically of IL-1 and TNF- α , keratinocytes, macrophages, T-cells, endothelial cells and fibroblasts in OLP lesions release significant amounts of IL-8. Interleukin-8 results in greater infiltration of T-cells such as cytotoxic T-cells at the site of OLP. Thus, IL-8 may be involved in the pathogenesis of OLP.^[11]

Studies have shown that serum levels of IL-6 and IL-8 are higher in OLP patients compared to healthy individuals.^[11,12] Moreover, serum level of IL-8 is a more specific indicator of OLP than IL-6.^[11]

Diabetes mellitus (DM) is a metabolic disease caused by impaired insulin production or resistance to it, and is characterized by the abnormal metabolism of glucose, proteins and lipids.^[13] In fact, type II DM is an inflammatory disease and inflammatory cytokines are involved in its pathogenesis.^[14] Serum level of IL-8 in type II DM is significantly higher than that in healthy individuals.^[15,16]

At present, a correlation between DM and OLP seems probable.^[17] The Prevalence of DM in OLP patients has been reported as 1.6% - 85%.^[18-20] Studies in this regard have been variable in the methodology and results. Jolly showed that 85% of patients with OLP in his study had abnormal glucose tolerance test results.^[21] Seyhan *et al.*, in their study stated that half of the patients with OLP had glucose metabolism impairment and one-fourth of these patients had DM.^[22] However, other studies failed to show a higher incidence of abnormal glucose tolerance test in OLP patients compared to the healthy population.^[23,24]

Since IL8 is an important inflammatory mediator involved in both conditions, the first objective of this study was to measure and compare the serum level of IL8 in four groups of OLP, DM, OLP+DM and healthy control. Considering the existing controversies regarding the possible association of DM and OLP, the second objective of this study was to assess the fasting blood sugar (FBS) and 2-h postprandial blood sugar in OLP patients.

MATERIALS AND METHODS

This analytical cross sectional study was conducted on three groups of OLP, type II DM and control.

Considering the 80% power of the study, $d = 50$ and $\alpha = 0.05$, the sample size was calculated to be 35 in OLP, 20 in type II DM and 20 in control groups. Samples were selected through convenience sampling among those presenting to Isfahan School of Dentistry and clinics in the city of Isfahan. The OLP group was divided into two subgroups of OLP alone and OLP + type II DM. Thus, 30 OLP, 5 OLP and type II DM, 20 type II DM and 20 healthy control individuals were evaluated. The healthy controls were selected among those presenting for check up and had no history of systemic disease or drug intake. An internal medicine specialist confirmed the health of control subjects. The DM patients were selected among subjects suspected for DM, who referred to a diabetes treatment center for a definite diagnosis. These subjects had FBS ≥ 110 mg/dL or 2-h postprandial blood sugar ≥ 200 mg/dL and reported polyphagia, polydipsia and polyuria.^[25] These subjects had no other systemic disease and reported no drug intake. The diagnosis of type II DM was made by an internal medicine specialist.

The OLP patients were selected among those presenting to Isfahan School of Dentistry and clinics in the city of Isfahan. Following observation of white striations and red streaks and inclusion of OLP in the list of differential diagnoses (according to the clinical criteria) by an oral medicine specialist, a biopsy sample was taken from the lesion. Upon confirmation of OLP by pathologist according to the World Health Organization criteria,^[26] the patient was included in the study. Patients with lichenoid reactions due to medications or contact and those with graft versus host disease (frequently Bone marrow graft) or cutaneous lesions were excluded. Fasting and 2-h postprandial blood sugar tests were requested for subjects with definite diagnosis of OLP. Based on the test results, they were assigned to OLP or OLP+DM group until 35 samples were recruited.

All participants underwent a thorough physical examination to ensure the absence of inflammation in other parts of the body. Tobacco users, alcohol consumers and patients with periodontitis were excluded. In general, patients with a history of systemic conditions other than DM and OLP and those on any type of medication were excluded from the study.

After 8 h of fasting, 3cc of blood was drawn from each subject between 7 and 9 a.m. (to prevent errors due to the circadian rhythm). Within 5-10 minutes, subjects had a usual breakfast (carbohydrate-rich) and underwent 2-h postprandial blood sugar test

two hours after the breakfast. Within this 2-h period, subjects were requested to refrain from severe physical exercise. Immediately after clotting, blood samples were centrifuged at 3000 rpm for 15 minutes to isolate the serum. Immediately afterwards, the FBS and 2-h postprandial blood sugar were measured. The remaining serum isolated from the fasting blood sample was stored at 20°C for measurement of IL-8 using the ELISA Kit (Quantikine ELISA, R and D Systems, Inc., USA). The obtained data were analyzed using SPSS (IBM Statistics 20), one-way ANOVA and post – hoc least significant difference (LSD) test. Normality of the data was checked using Kolmogorov- Smirnov test.

RESULTS

A total of 20 DM, 20 healthy control and 35 OLP individuals were included; 14.28% of OLP patients had type II DM. Furthermore, 20% of OLP patients had impaired fasting glucose (FBS of 100- 125 mg/dL).

As seen in Table 1, the highest mean concentration of serum IL-8 was seen in OLP+DM group followed by OLP, DM (6.1 ± 1.91 pg/mL) and control (5.3 ± 1.23 pg/mL) groups.

One-way ANOVA showed significant differences among groups in terms of serum level of IL8 ($P = 0.011$). Post-hoc LSD test was then applied and showed a significant difference in serum level of IL-8 between the control and OLP groups ($P = 0.002$). The difference between the OLP+DM and control subjects was also significant in terms of serum level of IL-8 ($P = 0.022$). However, although the mean serum level of IL-8 in the DM group was higher than that in the control group, this difference was not significant ($P = 0.087$). The serum level of IL8 in OLP+DM patients was higher than that in DM group but this difference was not significant either ($P = 0.219$). The serum level of IL8 was higher in OLP+DM group compared to the OLP group but not significantly ($P = 0.615$).

The mean levels of FBS and 2-h postprandial blood sugar in OLP+DM patients were the highest followed by DM, OLP and control groups, respectively [Table 2].

DISCUSSION

To the best of the authors' knowledge, no previous study has compared FBS, 2-h postprandial blood

Table 1: The mean (\pm standard deviation) and range of changes in serum interleukin-8 concentration in the four groups and result of *post hoc* least significant difference test

Group	Mean \pm SD (pg/mL)	Range of changes
Oral lichen planus	6.66 \pm 1.25 ^a	4.2-9.2
DM	6.1 \pm 1.91 ^{abc}	3.4-9.8
Oral lichen planus + DM	7.02 \pm 1.43 ^{ab}	5.2-8.5
Control	5.3 \pm 1.23 ^c	2.7-6.9

Same alphabet means no statistically difference between two groups. SD: Standard deviation; DM: Diabetes mellitus

Table 2: The mean levels of fasting blood sugar and 2-h postprandial blood sugar in the four groups

Group	FBS (mg/dL)	2-h postprandial blood sugar (mg/dL)
Oral lichen planus	14.4 \pm 99.85	36.92 \pm 109.95
DM	26.6 \pm 152.8	44.8 \pm 210.2
Oral lichen planus + DM	49.3 \pm 159.25	62.2 \pm 224.35
Control	12.01 \pm 92.83	32.2 \pm 104.56

FBS: Fasting blood sugar; DM: Diabetes mellitus

sugar and IL-8 levels in the serum of OLP, type II DM, OLP+DM and control subjects simultaneously; thus, the current study is probably the first one.

The first objective of this study was comparing the serum level of IL-8 in OLP subjects and healthy individuals. The results showed that the mean serum level of IL-8 in OLP patients was significantly higher than that in controls ($P < 0.05$). Sun *et al.*,^[11] in 2005 concluded that the serum level of IL-8 was significantly higher in OLP patients than controls and thus, IL-8 is a more sensitive marker than IL-6 for diagnosis of OLP. Rhodus *et al.* in 2005^[27] and Zhang *et al.* in 2008^[28] stated that the serum and salivary levels of IL8 were significantly higher than those in control group. Our findings are in agreement with the results of the above-mentioned studies. The following explanation can better elucidate the reason behind higher serum level of IL-8 in OLP patients compared to healthy controls. In general, there is a strong theory that T-cell-mediated autoimmune reactions are involved in the pathogenesis of OLP. The local and systemic release of different cytokines from the oral mucosa and blood is responsible for initiation and progression of this disease.^[29] Histological analysis has shown band-like infiltrates of T lymphocytes in the upper lamina propria of OLP lesions.^[30] Inflammatory cytokines such as TNF- α and IL-1 stimulate T - cells, monocytes, macrophages, keratinocytes, neutrophils, and endothelial cells and result in secretion of IL-8 in the lesion and in

the peripheral blood.^[31,32] Cell culture studies have shown that OLP keratinocytes produce higher levels of TNF- α than gingivitis keratinocytes.^[9,10] Thus, greater infiltration of inflammatory cells especially T - cells as well as increased secretion of TNF- α and IL-1 in these patients can lead to higher secretion of IL-8; which explains the high serum levels of IL-8 in OLP patients compared to healthy individuals.

Based on previous studies, OLP plus DM may occur concomitantly.^[18-20] Thus, the second objective of this study was to assess the serum level of IL-8 as a possible relevant factor in DM patients compared to healthy individuals. In the current study, the mean serum level of IL-8 in DM patients (6.1 ± 1.91 pg/mL) was higher than that in controls (5.3 ± 1.23 pg/mL); however, this difference was not significant ($P > 0.05$). According to a study by Abou-Shousha *et al.*,^[14] serum level of IL-8 in DM patients was significantly higher than that in healthy controls ($P = 0.032$). They suggested that serum level of IL-8 could be used as a predictor of DM-related micro- and macro-vascular diseases especially in high-risk individuals. Other studies also showed significantly higher serum level of IL-8 in type II DM compared to healthy individuals.^[15,16] Although in the above-mentioned studies the serum level of IL-8 in diabetic patients was significantly higher than that in controls, this difference in our study was not significant. Such difference in results might be due to the variable sample sizes, differences in the nutritional regimens of healthy individuals, genetic variations and ethnic characteristics of subjects in different geographical locations. In general, it seems that high glucose level in diabetic patients results in attachment of monocytes to endothelial cells and subsequent production of IL-8.^[15,33]

The third objective of the current study was to measure the serum level of IL-8 in patients with OLP+DM in comparison with OLP plu DM patients as well as healthy controls. In our study, a significantly higher level of serum IL-8 was found in OLP+DM patients compared to healthy controls (despite the small sample size of the former group)-($P < 0.05$). No previous study has been conducted in this regard to compare with.

Since the occurrence of DM and glucose impairments has been reported in OLP patients,^[18-20] the fourth objective of this study was to measure the levels of FBS and 2-h postprandial blood sugar in OLP patients

in comparison with other groups. The mean level of FBS and 2-h postprandial blood sugar was the highest in OLP+DM patients followed by DM, OLP and control groups, respectively. In our study, 14.28% of OLP patients had DM and 20% had impaired fasting glucose (FBS of 100-125 mg/dL). Despite the higher mean level of FBS in OLP patients compare to controls, this difference did not reach statistical significance.

Seyhan *et al.*^[22] found a significant difference in FBS between OLP and control subjects; whereas, some other studies failed to show a higher frequency of abnormal glucose tolerance test in OLP patients compared to the general population.^[23,24] Ansar *et al.*^[34] reported that the mean FBS in OLP patients was 102.5 ± 32 mg/dL. This value was close to the value reported in our study (99.85 ± 14.4 mg/dL). Such controversy in the results of the afore-mentioned studies may be due to different methodology and study designs. Higher FBS levels in OLP patients compared to healthy controls points to the possible role of glucose intolerance in the pathogenesis of OLP.^[35] In addition, the ascending trend of the serum level of IL8 in control, DM, OLP, and OLP+DM patients may indicate the role of this factor in the pathogenesis of OLP and DB; moreover, it may have a synergistic effect on both conditions.

In this study, it was not possible to detect internal inflammation (if any); thus, the IL-8 value was not exclusively indicative of OLP or DM. Small sample size in the OLP+DM group was another limitation of our study which was due to the small number of OLP patients and difficulty in encouraging them to participate in the study. Future studies with larger sample sizes are required on OLP patients to compare the serum level of IL-8 in different types of OLP and also to assess its role in the clinical course of the disease.

CONCLUSION

The results showed higher serum levels of IL-8 in OLP+DM and OLP patients compared to healthy controls. Thus, IL-8 should be considered as an effective inflammatory factor in the pathogenesis of this disease. Administration of medications balancing the level of IL8 and decreasing its serum level may be a step forward to alleviate the symptoms of OLP and OLP+DM patients.

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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 non-financial in this article.

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