# Effects of Intralesional Platelets-Rich Plasma Injections on Oral Lichen Planus Lesions and Salivary Interleukin-8

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## Abstract

**Background:** Oral lichen planus (OLP) is a chronic inflammatory disease of oral mucosal surfaces. **Objective:** The aim of this study was to determine if salivary IL-8 levels changed after autologous platelet-rich plasma (PRP) therapy and to assess its therapeutic effects on OLP. **Materials and Methods:** For each patient, demography, social, medical, and medication history was recorded. Before receiving 0.5 mL of PRP for each square centimeter of lesion, each patient was examined for phenotype, color, size, and site of OLP lesions. Patient's salivary samples were taken between 8 and 11 AM. Three to four milliliters of saliva was obtained from each patient. ELISA kit for IL-8 using a sandwich-ELISA technique, to measure salivary IL-8 before and after PRP injections. Each patient had signed a consent form to participate in this study. **Results:** Thirteen OLP patients took part in this study, six males (46.2%) and seven females (53.8%). Patients were between 32 and 79 years of age, with a mean age of 60.2 ± 13.9 years. Mean salivary IL-8 was (459.94 ± 233.74 pg/mL) before PRP injections and (465.68 ± 158.30 pg/mL) after PRP injections with no significant difference; however, IL-8 was higher after PRP injections. No association was found in salivary IL-8 level in relation to color changes, signs, and symptoms; pain and burning sensation, lesion phenotype, size, and location. **Conclusion:** Majority of OLP lesions showed an increased salivary IL-8 level after PRP treatment. PRP injections relieved OLP lesions' signs and symptoms, and turned hyperemic lesions into normal mucosal color, but lesions' dimensions were resistant to change.

**Keywords:** Interleukin 8, oral lesion, oral lichen planus, platelets-rich plasma

## Introduction

Oral lichen planus is a chronic inflammation of the oral mucosae, and its pathogenesis is related to aberrant cellular immunity,[1] Genetics, psychological state, and infectious agents may act as causes and/or triggers.[2] It was found that all the possible causes of OLP lesion production have the capacity to modulate the oxidative status,[3] and this is approved by the decreased salivary anti-oxidant capacity in OLP patients,[4] as antioxidants can neutralize OLP-inducing oxidants.[5] This disease worsens the life quality of patients, especially when erythematous erosive/ulcerative or erythematous phenotypes are manifested.[6] IL-8 is a vital mediator of the host's response to inflammation and damage. The activation of neutrophil chemotactic factor, neutrophils, basophils, and T cells are the functions of this cytokine. The production of IL-8 is carried out by a wide range of cells, such as neutrophils, fibroblasts, keratinocytes, endothelial cells, monocytes/macrophages, and T cells.[7] Interleukin-8 is a difficult cytokine to detect in healthy tissues, but pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) or interleukin-1 beta (IL-1β), as well as viral or bacterial agents, as well as cellular stress, may induce its production.[8] Numerous diseases, including as adamantias disease, unstable coronary artery disease, and newborn infections, may be monitored by measuring the levels of IL-8 in the blood.[9] In OLP, it's still unknown how significant is the level of IL-8 as a disease marker. OLP keratinocytes generate both tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1).[10] Also, peripheral blood mononuclear cells and

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tissue-infiltrating mononuclear cells from oral mucosal lesions of OLP both have the capacity to produce TNF-α.[11] OLP lesions with a wide range of cell types, including keratinocytes, endothelial cells, macrophages, fibroblasts, and T cells.[12] When induced by TNF-α and IL-1, each of these several cell types has the capacity to produce IL-8. It is possible that IL-8 will draw more T cells, especially cytotoxic T cells, to OLP lesions,[13] and as a result, it's believed that cytokines including IL-8 are having a role in the etiology of OLP.[14] Platelet-rich plasma was defined as condensed plasma with high concentration of platelet-derived growth factors (PDGF).[15] PRP with its anti-inflammatory properties aids to decrease the inflammation as well as accelerate the healing process,[16] and even bone regeneration.[17] PRP autologous characteristics lower the incidence of complications or side effects that may be experienced because it is extracted from the patient's own blood.[18] Platelets-rich plasma has the power to modify T-cell-mediated immunity, boost IL-2 and IFN activity, and restore phagocytosis in neutrophils and macrophages,[19] so PRP may have the capacity to modulate salivary IL-8 levels in patients with OLP.

## Materials and Methods

In this clinical trial, each patient with OLP provided their informed consent to participate in this study in accordance with ethical standards. The disease was either clinically (reticular phenotype) or clinically and histopathologically diagnosed (erosive and ulcerative types). Diagnosed patients were contacted to participate in this study. Patients who did not respond to any previous treatments were included in this study. Histopathological analysis was done at the department of oral pathology at the college of dentistry/ university of Baghdad. This study was carried out between February and June 2022 at the University of Baghdad's College of Dentistry, the Karbala Senior Care Home, and Babil Senior Care Home.

Clinical examination of patients was carried out preceded by recording demographic information name, age, gender, and occupation), as well as information regarding their medical, medication, family, social history, and OLP lesions (site, size, color, signs, and symptoms). Starting from the upper and lower labial mucosa and vestibule, the labial commissures and buccal mucosa, the tongue (dorsal, ventral surfaces, and margins), the hard and soft palate, and the gingiva and alveolar ridges. A WHO periodontal probe was used to measure the dimensions of each lesion, and images were taken to evaluate the color of the lesions both before and after PRP treatment.

Saliva was collected twice from each participant before and after the PRP treatment at the same time, between 8:00 and 11:00 A.M, to avoid the possibility of errors due to salivary circadian rhythm. The participants in the study were given instructions to abstain eating and drinking an hour preceding up to the collection of their saliva. Approximately 3–4 milliliters of unstimulated saliva was collected in graduated tubes. Saliva samples were stored in a deep freezer at a temperature of –80 C° until analysis of IL-8.

To prepare platelets-rich plasma, the antecubital vein was used for blood withdrawal with a syringe of 21 gauge. After that, the blood sample was placed into the PRP vacuum tube. The protocol for the preparation of PRP was carried out in accordance with Mazzocca 2012.[20] Following the collection of blood, the PRP tube was carefully blended and platelets can be concentrated after the cellular components have been separated using centrifugation at 3200 rpm for 15 minutes. To eliminate the majority of platelets-poor plasma (PPP), using a spinal needle with a gauge of 22 and removing two-thirds of the plasma layer. PRP can then be prepared for injection by aspirating it using a sterile insulin syringe of 1 milliliter capacity.

Following a field block with a vasoconstrictor-containing local anesthetic, the injections were administered to the lesions of OLP. The affected region received an injection with 0.5 milliliters of platelet-rich plasma for every squared centimeter of surface area.[21] Platelet-rich plasma injections were scheduled to be administered on a weekly basis for a total of three consecutive weeks as part of the treatment plan for each case. Four weeks after the last PRP session, the follow-up visits were planned. During these visits, the signs and symptoms of the patient were discussed, and the dimensions of each lesion were measured. The second saliva samples were obtained during the last follow-up visits to study the salivary IL-8 level after PRP treatment.

Sandwich ELISA is the technique that is utilized by the ELISA kit of IL-8 estimation. The optical density was calculated by the use of spectrophotometry at a wavelength of 450 ± 2 nm using a ChroMate® ELISA Reader. There is a one-to-one correspondence between the amount of human IL-8 present and the optical density. By comparing the optical density of each sample to the standard curve, we were able to ascertain the quantity of human IL-8 that was contained inside each sample.

Statistical analysis using SPSS-28(Statistical Packages for Social Sciences- version 28) was done. Frequency, percentage, mean, standard deviation, and range were utilized. Student's *t* test was used for making comparisons between two independent means. Paired *t* test was used for making comparisons between two dependent means. Analysis of the variance test was used for making comparisons between more than two independent means. The significance of difference of different percentages (qualitative data) was tested using Pearson chi-square test ($\chi^2$-test) with application of Yate's correction or Fisher Exact test whenever applicable. The statistical significance was considered when *P* value was less than 0.05.

## Ethical approval

The study was conducted in accordance with the ethical principles in the Declaration of Helsinki. It was carried out with patient's verbal and analytical approval. The study protocol and the subject information and the consent form were reviewed and approved by a local ethics committee according to the document number 751 (including the number and the date on December 12, 2022.

# Results

The ages of the patients were from 32 to 91 years, with a mean age of 60.2 years ±13.9 years. The majority of the patients were above the age of 60 years (61.5%), and 38.5% were below 60 years of age [Table 1].

Regarding the occupation, the majority of them were housewives (30.8%), followed by military officers (23.1%), then teachers, free workers, and retired patients (15.4%) each.

**Table 1: Age and gender of the study sample**

| | | No | % |
|---|---|---|---|
| Age (years) | <60years | 5 | 38.5 |
| | =>60years | 8 | 61.5 |
| | Mean±SD (Range) | 60.2 ± 13.9 (32-79) | |
| Gender | Male | 6 | 46.2 |
| | Female | 7 | 53.8 |

**Table 2: Mean salivary intrleukin-8 before and after platelet-rich plasma injections**

| | Before | After | P value |
|---|---|---|---|
| IL8 (pg/mL) | 459.94 ± 233.74 (77.65-987.66) | 465.68 ± 158.30 (243.01-821.55) | 0.928 |

-Data were presented as Mean±SD (Range)

#Significant difference between two dependent means using Paired-t-test at 0.05 level

## Social/ medical history

The majority of patients were with diabetes mellitus, followed by hypertension, cardiovascular disorders, and respiratory problems. Eight patients were with a history of medications for their systemic diseases, five patients were smokers, and two of the participants were former alcoholics.

## Interleukin-8 level, before and after PRP injections

Before and after treatment, the salivary levels of interleukin-8 were assessed in each patient and the levels found to be higher after PRP treatment [Table 2]. In relation to the location of the lesion, its size, the phenotype of the lesion, color change, and signs or symptoms that the patient was experiencing the levels of IL-8 were compared. It was found that there was no statistically significant relation between the estimated amount of IL-8 and the location of the lesions, their phenotype, dimensions, signs and symptoms, and color change that took place before and after treatment.

## Dimensions and color of OLP lesions after PRP injections

The OLP lesions in this study were of varying dimensions. After PRP injections, three lesions showed dimensional changes, which accounts for 10.3%, while the size of 26 lesions remained the same, which accounts for 89.7%. All 29 OLP lesions showed hyperemia at the first presentation. After receiving PRP, 27 of the lesions changed back to normal color, which accounts for 93.1%, while just two lesions remained hyperemic, which accounts for 6.9% of the total [Figure 1].

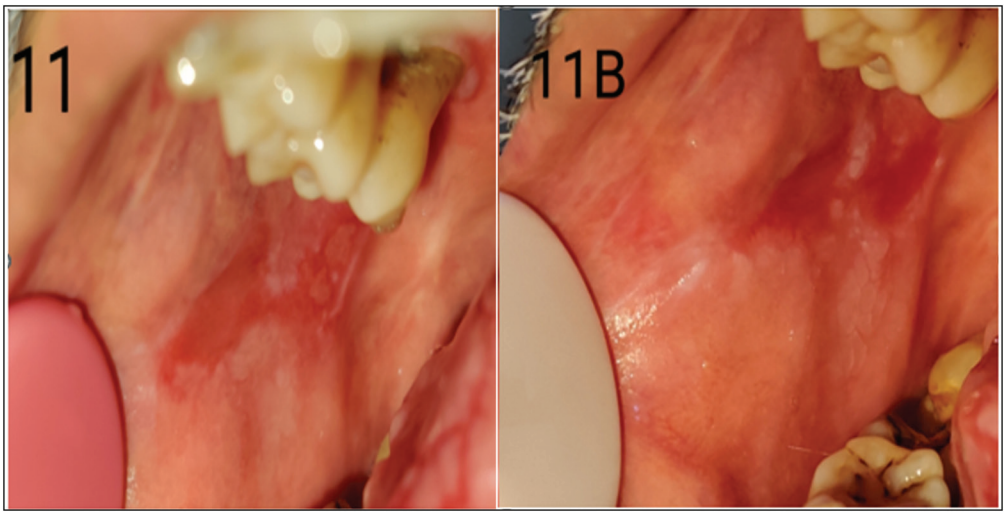


**Figure 1:** 11) lesion of OLP before treatment - patient No. 11. 11 B) lesions of OLP after treatment - patient No. 11. This figure illustrates a comparison between two status in the same patient that suffers from oral lichen planus; firstly prior to PRP treatment and secondly after PRP treatment

### Signs and symptoms of OLP lesions

Before PRP treatment, twelve of the lesions showed no symptoms (41.4%), whereas the other seventeen lesions did (pain/ burning sensation) (58.6%).

Following PRP injections, 24 of the lesions were found to be symptoms free, accounting for 82.8% of the total, and this was statistically of high significance ($P = 0.001$). Also, all the ulcerative lesions showed either ulcer disappearance or regression; however, five of the lesions showed no response to treatment (17.2%) [Table 3].

### Interleukin-8 in relation to phenotype and site of OLP lesions

Salivary IL-8 was assessed in each patient in relation to the location and phenotype of the lesion. Salivary IL-8 showed no significant association in relation to phenotype and location of the OLP lesions before and after PRP injections [Table 4].

### Interleukin-8 in relation to dimensions, color change, and signs and symptoms of OLP lesions

Salivary IL-8 showed no significant association in relation to neither size nor color change of the OLP lesions before and after PRP injections. However, salivary IL-8 levels in relation to signs and symptoms showed a significant association [Table 5].

**Table 3: Signs and symptoms of OLP lesions before and after PRP treatment**

| | | No | % |
|---|---|---|---|
| S and S Before | Pain, Burning | 17 | 58.6 |
| | No | 12 | 41.4 |
| S and S After | Pain, Burning | 5 | 17.2 |
| | No | 24 | 82.8 |
| | P value | 0.001* | |

*Significant difference between proportions using Pearson Chi-square test at 0.05 level

## Discussion

In this study, patients with recalcitrant OLP lesions which didn't respond to any previous treatment were given intralesional PRP injections for three consecutive weeks (an injection per week). According to many studies, females were more affected by OLP than males,[23-25] which agrees with this study. The majority of the patients were above the age of sixty (61.5%), and this was in accordance with a study.[26] Most of the patients in this study were with diabetes mellitus either alone or with hypertension, and this goes with the results of some studies.[27,28] All symptomatic lesions (pain/burning) and most of the hyperemic ones that were included in this study showed significant improvement, and this finding coincides with several previous studies,[4,21,22] which also used PRP injections for the treatment of OLP. Approximately 10.5% of lesions in this study showed reduction in size, while 89.5% showed no changes in size, and this might suggest more PRP sessions to achieve lesion size reduction, as in the studies that gave PRP injections at weekly intervals for at least eight weeks to get lesion size reduction.[21,22] Salivary IL-8 level in OLP patients was estimated in this study, and the results were compared before and after treatment with PRP injections. According to the results of a recent study, OLP patients showed a mean salivary level of IL-8 that was noticeably higher than the levels of the control group.[1] The elevated levels of cytokines seen in the saliva of OLP patients may be the result of an increase in the production of these cytokines by inflammatory cells or keratinocytes.[1,4,29] Up to our knowledge, no study estimated salivary IL-8 before and after the treatment of OLP; however, there was a study that compared serum levels of IL-8 before and after the treatment; this study concluded that the levels were lower after treatment.[30] The findings of this study indicated a mean level of salivary IL-8 that was higher after the treatment than was before, and this result might indicate more PRP sessions for those patients. In this study, the levels of salivary IL-8 were evaluated before and after therapy in

**Table 4: Interleukin-8 relation to lesion site and phenotype before and after PRP injection**

| | | No | IL8 (pg/mL) | | P value |
|---|---|---|---|---|---|
| | | | Before | After | |
| **Site** | Buccal mucosa | 20 | 474.09 ± 234.26 | 493.24 ± 146.90 | 0.707 |
| | Tongue | 4 | 425.03 ± 169.70 | 376.64 ± 102.05 | 0.629 |
| | Palatal mucosa | 2 | 324.57 ± 171.33 | 371.71 ± 133.74 | 0.327 |
| | Maxillary ridge | 2 | 445.72± | 466.28± | - |
| | Mandibular ridge | 1 | 399.67± | 397.20± | - |
| | P value | | 0.908 | 0.478 | |
| **Lesion Phenotype** | Reticular | 15 | 512.68 ± 246.93 | 522.15 ± 151.91 | 0.889 |
| | Ulcerative | 13 | 387.09 ± 141.42 | 401.16 ± 90.67 | 0.648 |
| | Erosive | 1 | 399.67± | 397.20± | - |
| | P value | | 0.279 | 0.054 | |

#Significant difference between two independent means using Student *t* test at 0.05 level

#Significant difference between two dependent means using Paired *t* test at 0.05 level

^Significant difference among more than two independent means using ANOVA test at 0.05 level

**Table 5: Interleukin-8 in relation to lesion dimensions, color change, and signs and symptoms before and after PRP injection**

| | | No | IL8 (pg/mL) | | P value |
|---|---|---|---|---|---|
| | | | Before | After | |
| **Dimensions, Before** | 1x1 | 2 | 359.94±221.35 | 466.70±268.07 | 0.191 |
| | 1x2 | 4 | 511.92±171.90 | 464.23±54.41 | 0.508 |
| | 2x2 | 3 | 671.60±292.53 | 406.80±112.68 | 0.183 |
| | 1x3 | 3 | 441.88±6.65 | 465.18±1.90 | 0.014# |
| | 2x3 | 11 | 446.67±235.34 | 506.51±183.84 | 0.434 |
| | 3x3 | 1 | 445.72± | 466.28± | - |
| | 1x4 | 2 | 187.68±155.60 | 336.97±132.87 | 0.068 |
| | 3x4 | 3 | 426.53±23.97 | 442.16±38.97 | 0.237 |
| | P value | | 0.413 | 0.886 | |
| **Dimensions, follow-up** | Reduction | 3 | 284.19±139.89 | 340.19±109.20 | 0.087 |
| | No change | 26 | 471.91±207.12 | 477.84±134.67 | 0.885 |
| | P value | | 0.141 | 0.101 | |
| **Color change of the lesion** | Red | 2 | 457.24± | 821.55± | - |
| | Normal | 27 | 452.13±215.40 | 437.09±98.65 | 0.661 |
| | P value | | 0.974 | 0.001# | |
| **S and S Before** | Pain, Burning | 17 | 391.31±122.75 | 400.03±78.56 | 0.708 |
| | No | 12 | 539.16±271.62 | 553.66±154.77 | 0.866 |
| | P value | | 0.057 | 0.002# | |
| **S and S After** | Pain, Burning | 5 | 429.61±28.90 | 580.10±222.04 | 0.160 |
| | No | 24 | 457.25±228.40 | 439.33±104.06 | 0.643 |
| | P value | | 0.792 | 0.034# | |

#Significant difference between two independent means using Student *t* test at 0.05 level

#Significant difference between two dependent means using Paired *t* test at 0.05 level

^Significant difference among more than two independent means using ANOVA test at 0.05 level

relation to the lesion's location, size, phenotype, any color changes, and signs or symptoms the patient may have been experiencing. In this study, there was no statistically significant association between the estimated amount of IL-8 and the location of the lesions, their phenotype, size of the lesions, and the color change. Estimates of IL-8 both before and after treatment indicated a statistically significant association with signs and symptoms of the lesions. There are no studies that compared the levels of salivary IL-8 before and after treatment with PRP or any other type of treatment, while they only compared the levels among cases and controls.[1,30,31] According to the findings of this study, intralesional PRP injections may be a viable treatment option for OLP, and this is supported by two studies.[21,22] However, this need to be supported by other studies in the future with larger sample size, more PRP sessions, and longer follow-up periods.

## Conclusion

The use of intralesional PRP injections for OLP in this study showed promising results regarding alleviating pain and color change of the lesions into normal, and in turn improving the quality of life, especially in erythematous and erosive/ ulcerative phenotypes. Salivary IL-8 levels after the PRP treatment were found to be higher in most of the patients. In relation to signs and symptoms, the levels of IL-8 showed a significant association, while there was no significant association in relation to OLP lesion size, site, color change, and phenotype.


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## Conflicts of interest

There are no conflicts of interest.

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