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*Research Article*

# Salivary Interleukin-6 Level in Iraqi Patients with Oral Lichen Planus receiving Platelet-Rich Plasma Injections

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**ABSTRACT**

***Background:*** Interleukin-6 (IL-6) is a cytokine that has several functions, including stimulating growth and inhibiting cell death. It has the potential to operate as a biomarker for the accurate prediction of disease severity and activity, platelets-rich plasma was used in the treatment of oral lichen planus and can change the salivary IL-6 level.

**Objectives:** To study the clinical outcome of intralesional platelets-rich plasma in patients with oral lichen planus and to measure salivary IL-6 levels before and after the treatment with platelets-rich plasma were the aims of this study.

***Subjects and Methods***: In this clinical trial, for each patient a standardized case sheet was filled including demography, social, medical, and medication history. Before receiving, each patient was examined for phenotype, color, size, and site of oral lichen planus lesions. Patient's salivary samples were taken between 8 and 11 a.m. Three to four milliliters of saliva was obtained from each patient. The ELISA kit for IL-6 using sandwich-ELISA technique, to measure salivary IL-6 before and after PRP injections.

***Results***: Thirteen oral lichen planus patients were took part in this study, six males (46.2%) and seven females (53.8%). The patients were between 32 and 91 years of age, with a mean age of 60.2(±13.9) years. All symptomatic and most of the hyperemic ones showed improvement after PRP injections, while the size of the lesions was resistant to change. Mean salivary IL-6 was 44.27 pg/mL (±43.24) before PRP injections and (69.74±59.86 pg/mL) after PRP injections. No significant difference was found, however IL-6 was higher after PRP injections. In relation to color changes after PRP, there was a significant changes in IL-6 level compared with that before PRP injections. Similarly, a significant relation was found between IL-6 level and signs and symptoms; pain and burning sensation. No association was found in salivary IL-6 level in relation to lesion phenotype, size, and location.

***Conclusion***: All symptomatic OLP lesions that were treated with intralesional PRP responded very well, similarly almost all hyperemic lesions turned into normal mucosal color after completing the course of treatment. The majority of OLP lesions showed an increased salivary IL-6 levels after PRP treatment.

## Introduction

Oral lichen planus (OLP) is a chronic inflammatory lesion of the oral mucosae, its related to aberrant cellular immunity (1), Genetics, psychological state and infectious agents may act as causes and /or triggers (2). It's 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). This disease worsens the life quality of patients, especially when erosive/ulcerative or erythematous phenotypes are manifested (5). The cytokine Interleukin-6 (IL-6) plays a key role in the acute phase response and other immunological and inflammatory responses (6). Individuals with either severe cutaneous lichen planus or OLP exhibited higher amounts of IL-6 in their saliva (7). Researchers found that the amount of IL-6 identified in the saliva of OLP patients has fallen to a non-detectable level after different types of therapies (8). Platelet-rich plasma was defined as condensed plasma with high concentration of platelet-derived growth factors, such as: transforming growth factor-β, vascular endothelial growth factors (VEGFs), fibroblast growth factor (FGF), Epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), and hepatocyte growth factor (HGF) (9) . PRP with its anti-inflammatory properties aids to decrease the inflammation as well as accelerate the healing process (10). PRP autologous characteristics lowers the incidence of complications or side effects that maybe experienced because it is extracted from the patient's own blood (11). Recent research has shown that PRP, either alone or in conjunction with other treatments, has the power to change the level of IL-6 in the saliva of OLP patients (12). Salivary level of IL-6 maybe used as a reliable marker for evaluating the therapeutic effectiveness of medications in OLP patients (13). Peripheral blood mononuclear cells and tissue-infiltrating mononuclear cells from OLP lesions both have the capacity to make tumour necrosis factor-alpha (TNF-α). Also, OLP lesions having keratinocytes, macrophages, T cells, endothelial cells, and fibroblasts (14), these cells produce IL-6 in response to IL-1 and TNF-α stimulation (15). There is a chance that IL-6 may attract cytotoxic T lymphocytes to OLP lesions (16).

Platelets rich plasma has the power to modify T-cell-mediated immunity, boost IL-2 an interferon (IFN) activity, and restore phagocytosis in neutrophils and macrophages (17). According to patient surveys, those with erythematous or erosive/ulcerative OLP have a worse quality of life (18), receiving autologous PRP treatment may improve their quality of life. This study was conducted to assess PRP injections effectiveness in OLP treatment as well as the effects of PRP on salivary IL-6 levels.

## Subjects and Methods

After ethical approval OLP patients with informed consent were participated in this clinical trial. Previously diagnosed patients were contacted to participate in this study. Patients who did not respond to previous treatments were included in this study. Patients who had had any kind of OLP therapy during the past four weeks prior to sample collection, patients who had any kind of oral surgery or were injured during the four weeks before sample collection, and patients whose histological reports revealed the presence of dysplastic changes were excluded.

This study was carried out between February and June 2022 at the University of Baghdad/ College of Dentistry, Karbala Senior Care Home, and Babil Senior Care Home.

Clinical examination of patients was carried out using a disposable dental mirror, preceded by case sheet filling which contains demographic information name, age, gender, and occupation, as well as information regarding their medical, medication, family, social history, and OLP lesions (site, size, colour, phenotype, 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 (19). A WHO periodontal probe was used to measure the dimensions of each lesion, and images were taken to evaluate the colour and size of the lesions before and after PRP treatment. Considering saliva collection, it was collected twice from each participant, at first presentation and four weeks after the last PRP session at the same time, between 8:00 and 11:00 A.M, to avoid the possibility of errors due to salivary circadian rhythm (20). The participants in the study were given instructions to abstain eating and drinking an hour preceding the collection of their saliva(21). Approximately 3-4 millilitres of unstimulated saliva was collected. The saliva samples were stored in a deep freezer at a temperature of -80 C° until analysis of IL-6 (22).

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,etal. (2012) (23). 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 the 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 millilitre capacity (24).

Administration of platelets-rich plasma

Following a field block with a vasoconstrictor-containing local anaesthetic, the injections were administered to the lesions of OLP. The affected region received an injection with 0.5 millilitres of platelet-rich plasma for every squared centimetre of surface area(22). 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 re-measured (23). The second saliva samples were obtained four weeks after the third last PRP session to study the salivary IL-6 level after PRP treatment.

**Salivary interleukin-6 analysis**

Sandwich ELISA is the technique that is utilized by the ELISA kit of IL-6 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-6 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-6 that was contained inside each sample.

**Statistical analysis**

Using SPSS-28(Statistical Packages for Social Sciences- version 28); the statistical analysis was done. Frequency, percentage, mean, standard deviation, and range were utilized. Students' t-test was used for making comparisons between two independent means. Paired's t-test was used for making comparisons between two dependent means. Analysis of Variance test was used for making comparisons between more than two independent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test ( 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.

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 751 (including the number and the date in (28 / 12 / 2022) to get this approval.

## Results

**Demography**

In this study, a total of thirteen patients, with ages from 32 to 91 years, with a mean age of 60.2 years ±13.9. The majority (8 patients) of the patients were more than age of 60 years (61.5%), and five patients (38.5%) were below 60 years of age, with seven females (53.8%) slightly more males (6 patients) (46.2%); (Table 1).

**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 |

Regarding occupation, seven patients (53.8%)were workers and six (46.2%) were not. The majority of them (4 patients) were housewives (30.8%), followed by military officers (3 patients) (23.1%), then teachers, free workers, and retired patients (2 patients) (15.4%) each.

Oral lichen planus patients were mainly from Baghdad (5 patients) and Babil (5 patients) each accounted for 38.5% of the patients that participated in the study; the remaining patients were from Najaf, Karbala, and Wasit (only one patient from each of these provinces).

**Social/ Medical history**

The majority of patients were with diabetes mellitus, which accounts for 61.5%, followed by hypertension, which accounts for 53.8%, cardiovascular disorders, which account for 38.5%, and respiratory problems, which account for 15.4%. Eight patients were with a history of medications for their systemic diseases (61.5%), five patients were smokers (38.5%), and two of the participants were with alcohol drinking history (15.4%).

**Interleukin-6 level, before and after PRP injections**

Before and after treatment, the salivary levels of interleukin-6 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, colour change, and signs or symptoms that the patient was experiencing the levels of IL-6 were compared. It was found that there was no statistically significant relation between the estimated amount of IL-6 and either the location of the lesions or their phenotype. Estimates of IL-6 both before and after treatment indicated no differences that were statistically significant in relation to the extent of the lesion. Changes in IL-6 that were statistically significant were seen both before to the administration of PRP injections and during the subsequent assessment of signs and symptoms. According to the results of the statistical analysis, there was a statistically significant difference in the colour change that took place before and after treatment.

**Table 2:** Mean salivary intrleukin-6 before and after platelets-rich plasma injections

| | Before | After | P value |
|---|---|---|---|
| IL6 (pg/mL) | 44.27±43.24 (12.42-147.40) | 69.74±59.86 (10.00-183.71) | 0.197 |

-Data were presented as Mean±SD (Range)

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

**Dimensions and colour changes of OLP lesions after PRP treatment**

The OLP lesions in this study were between 1*1 to 3*4 centimetres. After PRP injections, three lesions out of 29 showed dimensional changes, which accounts for 10.3%, while the size of 26 lesions remained the same, which accounts for 89.7%. All the 29 OLP lesions showed hyperaemia at the first presentation. After receiving PRP, 27 of the lesions changed back to normal colour, which accounts for 93.1%, while just two lesions remained hyperaemic, which accounts for 6.9% of the total; Figure (1).

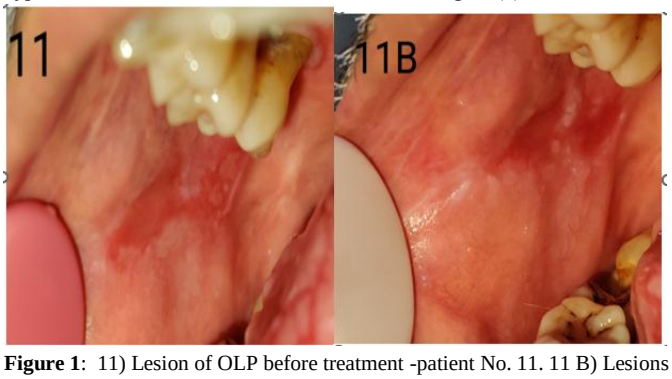


**Figure 1**: 11) Lesion of OLP before treatment -patient No. 11. 11 B) Lesions of OLP after treatment -patient No. 11

**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).

**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.

**Interleukin-6 in relation to phenotype and site of OLP lesions**

Salivary IL-6 were assessed in each patient in relation to the location and phenotype of the lesion. Salivary IL-6 showed no significant association in relation to phenotype and location of the OLP lesions before and after PRP injections; Table (4).

**Interleukin-6 in relation to dimensions, colour change, and signs and symptoms of OLP lesions**

Salivary IL-6 showed no significant association in relation to neither size nor colour change of the OLP lesions before and after PRP injections. However, salivary IL-6 levels in relation to signs and symptoms showed significant association; Table (5).

**Table 4**: Interleukin-6 relation to lesion site and phenotype before and after PRP injection.

| Oral lichen planus lesions | | No | IL6 (pg/mL) | | P value |
|---|---|---|---|---|---|
| | | | Before | After | |
| Site | Buccal mucosa | 20 | 39.97±39.89 | 69.87±59.63 | 0.032# |
| | Tongue | 4 | 63.50±62.10 | 66.09±64.68 | 0.966 |
| | Palatal mucosa | 2 | 84.56±88.88 | 18.90±1.02 | 0.482 |
| | Maxillary ridge | 2 | 21.71± | 18.18± | - |
| | Mandibular ridge | 1 | 63.40± | 130.40± | - |
| | P value | | 0.553 | 0.426 | |
| Lesion Phenotype | Reticular | 15 | 31.50±34.20 | 58.93±56.27 | 0.129 |
| | Ulcerative | 13 | 61.03±52.72 | 65.54±60.79 | 0.826 |
| | Erosive | 1 | 63.40± | 130.40± | - |
| | P value | | 0.208 | 0.502 | |

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

**Table 5**: Interleukin-6 in relation to lesion dimensions, colour change, and signs and symptoms before and after PRP injection

| Oral lichen planus lesions | | No | IL6 (pg/mL) | | P value |
|---|---|---|---|---|---|
| | | | Before | After | |
| Dimensions before PRP | 1x1 | 2 | 85.52±87.51 | 101.67±116.03 | 0.929 |
| | 1x2 | 4 | 53.37±46.30 | 79.60±74.17 | 0.220 |
| | 2x2 | 3 | 36.89±31.86 | 39.92±27.83 | 0.476 |
| | 1x3 | 3 | 52.69±53.65 | 64.11±79.55 | 0.525 |
| | 2x3 | 11 | 47.63±51.07 | 58.80±48.35 | 0.646 |
| | 3x3 | 1 | 21.71± | 18.18± | - |
| | 1x4 | 2 | 16.81±6.20 | 31.51±30.41 | 0.671 |
| | 3x4 | 3 | 32.25±27.44 | 101.51±73.29 | 0.246 |
| | P value | | 0.891 | 0.794 | |
| Dimensions after PRP | Reduction | 3 | 105.50±72.57 | 19.14±0.83 | 0.172 |
| | No change | 26 | 38.95±36.70 | 69.57±58.87 | 0.012# |
| | P value | | 0.012# | 0.156 | |
| Colour change | Red | 2 | 23.61± | 118.12± | - |
| | Normal | 27 | 47.48±46.02 | 60.37±57.94 | 0.328 |
| | P value | | 0.477 | 0.177 | |
| Signs and symptoms before PRP | Pain, Burning | 17 | 56.89±47.36 | 69.33±58.44 | 0.448 |
| | No | 12 | 30.17±37.18 | 57.31±58.64 | 0.224 |
| | P value | | 0.155 | 0.590 | |
| Signs and symptoms after PRP | Pain, Burning | 5 | 37.13±24.47 | 130.60±15.45 | 0.003# |
| | No | 24 | 47.65±48.12 | 50.56±53.58 | 0.824 |
| | P value | | 0.641 | 0.003# | |

#Significant difference between two independent means using Students-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.

## 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 with OLP than males (27,28,29), which agrees with this study. The majority of the patients were above the age of sixty (61.5%), and this was in accordance with Amadori, et al. (2017) (30). Most of the patients in this study were with diabetes mellitus either alone or with hypertension, and this goes with the results of Torrente Castells et al. (2010) and Ahmed et al., (2017) (31,32). All symptomatic lesion (pain/burning) and most of the hyperaemic ones that were included in this study showed significant improvement, and this finding coincides with several previous studies (5, 25, 26), 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 (25, 26). Salivary IL-6 level in OLP patients was estimated in this study, and the results compared before and after treatment with PRP injections. According to the meta-analysis that was done by Mozaffari, et al., (2018) (33), serum and salivary IL-6 levels were higher in OLP patients than controls, and the salivary levels where higher than serum in OLP patients. The elevated levels

of cytokines seen in the saliva of OLP patients maybe the result of an increase in the production of these cytokines by inflammatory cells or keratinocytes (1,5). Up to our knowledge, no study estimated salivary IL-6 before and after the treatment of OLP, however there were studies that compared serum levels of IL-6 before and after the treatment; these studies concluded that the levels were lower after treatment (34, 35). The findings of this study indicated that the mean level of salivary IL-6 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-6 were evaluated before and after therapy in relation to the lesion's location, size, phenotype, any colour 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-6 and the location of the lesions, their phenotype, size of the lesions, and the colour change. Estimates of IL-6 both before and after treatment indicated statistically significant in association with signs and symptoms of the lesions. There are no studies compared the levels of salivary IL-6 before and after treatment with PRP or any other type of treatment, while they only compared the levels among cases and controls (1,12). According to the findings of this study, intralesional PRP injections maybe a viable treatment option for OLP, and this is supported by Ahuja, et al., (2020) and Hijazi, et al., (2022) (25,26). 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 colour change of the lesions into normal, and in turn improving the quality of life, especially in erythematous and erosive/ ulcerative phenotypes. Salivary IL-6 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-6 showed significant association, while there was no significant association in relation to OLP lesion size, site, colour change, and phenotype.

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**Conflict of Interest**

Authors declare no conflict of interest.

**Data availability**

Data are available upon reasonable request.

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