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Effectiveness of platelet-rich plasma gel alongside tacrolimus ointment in managing erosive oral lichen planus and its effect on oral immune microenvironment

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Abstract: Oral lichen planus (OLP) is an autoimmune disease that significantly impacting patients' life quality. To investigate the clinical efficacy of platelet-rich plasma (PRP) gel coupled with tacrolimus ointment in managing erosive oral lichen planus (EOLP) and its effect on the oral immune environment, 80 EOLP patients were selected from 2021 to 2023 and randomised to two groups in our hospital. Both groups were given tacrolimus ointment, the study group added PRP gel. Comparative analysis of lesion area scores and overall treatment effects, wound pain index, salivary inflammatory factors and CD3+, CD4+, CD8+, CD4+/CD8+ ratio, immunoglobulin M (IgM) Changes, adverse reactions and relapses records. After treatment, compared to the control group, the overall effective rate of the study group was significantly higher, the lesion area and VAS score decreased, inflammatory factors decreased, and immune indexes improved (elevated CD3+, CD4+ and CD4+/CD8+ and a decrease in CD8+ and IgM) (P<0.05). Neither of them with serious adverse reactions (P>0.05). The study group's recurrence rate was lower than the control group's (P<0.05). This study demonstrated that PRP gel combined with tacrolimus ointment is superior to tacrolimus ointment alone for the treatment of EOLP and can be further applied to clinical treatment.

Keywords: Platelet-rich plasma gel, tacrolimus ointment, erosive oral lichen planus, immunization.

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INTRODUCTION

Oral lichen planus (OLP) is a prevalent chronic inflammatory condition among oral mucosal diseases, characterized by the epithelial basement membrane lymphocyte infiltration and cell liquefaction (Elenbaas *et al.*, 2022). This condition is more prevalent among middle-aged and elderly women (Li *et al.*, 2020), with typical clinical features including white streaks, erythema, and oral ulcers (González-Moles *et al.*, 2021). Among these, Erosive Oral Lichen Planus (EOLP) is rarer than other types. However, clinical practice indicates that EOLP exhibits more pronounced symptoms, is relatively challenging to treat and poses a higher risk of lesion progression to squamous cell carcinoma. Therefore, special attention should be given to patients with erosive EOLP (Didona *et al.*, 2022).

EOLP exhibits the properties of recurring nature and a protracted course, with the majority of patients enduring the illness span for numerous years. Patients experience pronounced discomfort, frequently resulting in difficulties with ingestion. Furthermore, due to its propensity for relapse and persistence, EOLP significantly impairs patients' quality of life (Ismail and Sinclair, 2020). Research indicates that corticosteroids, serving as the primary therapeutic modality for EOLP, alleviate pain and quell inflammatory responses, thus being considered the premier treatment option (Sridharan and Sivaramakrishnan, 2021). Nevertheless, prolonged corticosteroid administration elicits dosage-related adverse effects in patients, encompassing telangiectasia, localized mucosal atrophy and a predisposition to withdrawal rebound. Concurrently, a considerable proportion of EOLP patients possess contraindications to steroid hormone usage, such as diabetes, hypertension, and gastrointestinal ulcers (Kia *et al.*, 2020). Consequently, the pursuit of a targeted therapeutic agent that can effectively manage the onset and progression of EOLP while minimizing adverse reactions has emerged as a pivotal area of clinical and scientific investigation.

Tacrolimus is a non-steroidal immune modulator, categorized under calcineurin inhibitors. It has garnered attention in domestic and international literature for its safe and effective treatment of EOLP (Sun *et al.*, 2019). Nevertheless, during its administration, there can be localized adverse reactions reported, encompassing temporary sensations of burning, tingling, or alterations in taste. Although these side effects are generally mild and transient in nature, they tend to alleviate or diminish with time (Pinto *et al.*, 2023; Su *et al.*, 2022). Nevertheless, extended use of tacrolimus might increase the risk of mucosal co-infections and cancerous changes (Arana *et al.*, 2021). Consequently, a combined therapy approach is being contemplated as a means to mitigate its potential adverse consequences.

Platelet-rich plasma gel is prepared by platelet-rich plasma (PRP) activated by thrombin and calcium (Smith

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et al., 2022). Being abundant in bioactive components like growth factors and cytokines, PRP serves as a promising therapeutic alternative for managing EOLP (Sriram *et al.*, 2023). The bioactive constituents within this platelet-enhanced plasma foster cellular proliferation and differentiation, bolster angiogenesis and tissue remodeling, positively influence wound recovery and enhancing the oral immune niche (Jain and Gulati, 2016; Meznerics *et al.*, 2022). Numerous investigations have underscored the new role of platelet-concentrated plasma in accelerating wound healing processes, enhancing patients' quality of life while mitigating the economic strain of wound care. Huber *et al.* (2021) demonstrated the advantage of PCP for patients afflicted with Behcet's disease and oral ulcers, notably elevating T-regulatory cell counts and maintaining stable anti-inflammatory cytokine activity. Furthermore, several case series have highlighted the substantial therapeutic promise of PCP in EOLP treatment (Anitua *et al.*, 2022; Merigo *et al.*, 2018).

The present investigation aimed to contrast the lesion area pre- and post-treatment through the establishment of a comparative cohort. To evaluate wound pain severity prior to and following treatment, the visual analog scale (VAS) was utilized. Additionally, alterations in blood inflammatory markers and immunological parameters among patients were monitored. This research was to explore the clinical effectiveness of PRP gel combined with tacrolimus ointment for treating EOLP and to scrutinize its repercussions on the oral immune milieu. By adopting a comprehensive and rigorous scientific approach, we aspire to contribute novel insights and therapeutic strategies to clinical practice, thereby enhancing patient treatment outcomes and prognosis.

MATERIALS AND METHODS

Research object

2021 to 2023, a total of 80 individuals diagnosed with EOLP from the Department of Dental Medicine of Huaibei Miners General Hospital in Huaibei City, Anhui Province, were meticulously selected for this study. The age of the patients was 36 ~ 67 years old, and the course of disease was 3 ~ 36 months.

Diagnostic, inclusion and exclusion criteria

The diagnosis, inclusion and exclusion criteria of EOLP patients were performed with reference to the reports of Almutairi *et al.* (2023) and Rotaru *et al.* (2020).

Diagnostic criteria: (1) Clinical manifestations: linear, annular or network-like intricate pattern-like lesions formed by white and gray-white small papules, forming a typical Wickenham line; (2) Erosion type: often occur on the basis of hyperemia erosion and into hyperemia erosion type; (3) Histopathological criteria: lymphocytes in the lamina propria were banded or diffusely infiltrated, and the surface layer had excessive keratinization and incomplete keratinization.

Inclusion criteria: (1) Over the age of 18 years old, can cooperate with this study, no cognitive impairment or mental illness; (2) Patients diagnosed with EOLP according to EOLP diagnostic criteria; (3) The lesions only occurred on the back of the tongue and the buccal side (bilateral or unilateral) and there was no obvious abnormality in the rest of the oral mucosa; (4) those who can receive autologous blood treatment; (5) No immunosuppressive agents were used within 3 months.

Exclusion criteria: (1) Patients presenting with oral mucosal thickening or other oral mucosal conditions; (2) Patients with hematologic or immunologic disorders; (3) Patients with clotting abnormalities; (4) Patients with advanced periodontal disease and gingivitis; (5) Smoking and drinking within 3 months; (6) Can not follow the doctor's advice on time referral.

Preparation method of autologous PRP gel

The autologous PRP gel was prepared by referring to the method reported by Godoi *et al.* (2022) with simple modifications. According to the specific lesion area of the patient, 5 to 10mL of autologous blood was accurately extracted and then injected into a test tube pre-loaded with 1.0 to 1.5mL ACD anticoagulant to prevent blood coagulation. PRP was prepared by secondary centrifugation. At room temperature, a test tube containing blood is placed in a medical centrifuge to perform the first centrifugation (2200 rpm/min, 10 min). After centrifugation, the blood in the test tube will naturally separate into three distinct layers from top to bottom: a layer of platelet-poor plasma, a layer of platelet-rich concentrate and a layer of red blood cells. The platelet-poor plasma, platelet concentrate and approximately 2 mm of the red blood cell layer were collected for a second centrifugation (3000 rpm/min, 10 min). Remove the supernatant, the remaining liquid mixture. The concentrated platelets were resuspended in the test tube and the obtained suspension was PRP. 10 % calcium gluconate sodium chloride injection was mixed with thrombin powder to make a mixture of 1000 U/mL. The mixture was mixed with PRP at a ratio of 1:10 and the gel was formed after 30~50 s, that is, PRP gel.

Treatment methods

Eighty individuals diagnosed with EOLP were randomly divided into a study group and a control group, consisting of 40 patients in each group. The treatment duration was four weeks.

Control group: 0.03% tacrolimus ointment was used for local medication at the lesion site, once a day in the morning and evening.

Study group: In the control group on the basis of the treatment, PRP gel was applied to the clean and dry affected area (the gel should cover all erosive surfaces, twice a week for four weeks as the treatment course).

Do not swallow after smearing and drink and eat after 30 min. Patients in each group maintained a normal and non-irritating daily diet and good oral hygiene habits. The lesion range, erosion degree and overall condition of patients before and after treatment were evaluated.

Efficiency evaluation

The objective index signs and subjective symptom pain scores of patients before and after 4 weeks of treatment were recorded. Objective index signs: according to the size of the lesion area and the degree of mucosal congestion from light to heavy score (Hettiarachchi *et al.*, 2017): 0 was divided into no lesion and normal mucosa; 1 was divided into light white stripes, no congestion, or erosion surface; 2 were divided into white stripes with congestion area <1cm^2; 3 were divided into white stripes with hyperemia area >1cm^2; 4 were divided into white stripes with erosion surface <1cm^2; 5 were divided into white stripes with erosion surface >1cm^2. Subjective symptom pain score: VAS table score (Ozdemir *et al.*, 2020): A score of 0 means no pain at all; a score of less than 3 represents mild pain; scores of 4 to 6 represent significant pain; a score of 7 to 10 represents the presence of intense pain.

According to the EOLP efficacy criteria in "Diagnosis of oral lichen planus" (Gururaj *et al.*, 2021), the clinical efficacy after 4 weeks of treatment was evaluated: (1) Markedly effective: a. objective indicators: congestion and erosion completely disappeared after treatment, white stripes were no or mild (signs were scored as 0 or 1 points). b. Subjective indicators: pain disappeared completely (symptom score was 0). (2) Effective: a. objective indicators: After treatment, the area of congestion and erosion decreased, and the white stripes decreased (the score of signs decreased). b. Subjective indicators: pain relief (decreased symptom score). (3) Ineffective: a. objective indicators: after treatment, the area of hyperemia and erosion did not change or increase, and the white stripe did not change or increase (the score of signs did not change or increase). b. Subjective indicators: no relief or aggravation of pain (symptom score unchanged or increased). All patients were evaluated 4 weeks after treatment.

The safety of treatment was evaluated according to whether complications or side effects occurred during the treatment. All patients with significant effects were regularly reviewed. Within 12 weeks after the end of the treatment, if the clinical symptoms reappeared or gradually aggravated, it was considered a recurrence.

Detection of salivary inflammatory factors and blood immune cells

Referring to the method reported by Alarcón-Sánchez *et al.* (2024) to detect the level of inflammatory factors in the salivary glands of patients. Saliva (2 mL) was gathered from all patients the day prior to treatment and one day after the completion of four weeks of therapy. The supernatant was obtained through centrifugation at room temperature (3000 rpm/min, 15 min). The levels of tumor necrosis factor-α (TNF-α), interleukin 17 (IL-17), and interleukin 6 (IL-6) in the saliva were measured using ELISA. The kits were supplied by Shanghai Enzyme-linked Biotechnology Co., Ltd., with the following numbers: ml077385, ml062891 and ml058097.

The immune index of blood cells was assessed using flow cytometry (Ma *et al.*, 2023): 2mL of venous blood was collected from all patients the day before treatment and one day after the four-week treatment period. To anticoagulate, 100μL of whole blood was mixed with 30μL of heparin sodium solution; then, 100μL of the anticoagulated blood was combined with fluorescently labeled monoclonal antibodies CD3+, CD4+, and CD8+, and incubated in the dark for 30 min. Add red blood cell lysate, mix by blowing, and in the dark for 10 min. The solution centrifugal (1500 rpm/min, 5 min), abandon the supernatant. After washing with PBS, the supernatant was discarded again after a second centrifugation (1500 rpm/min, 5 min). Analyzed using flow cytometry.

Blood immunity indices of immunoglobulin M (IgM) were evaluated using the immunoscattering turbidimetric technique according to the method of Djukić *et al.* (2024). 2 mL of venous blood was collected from all patients the day before treatment and one day after the four-week treatment period. Following serum separation, IgM levels were evaluated in accordance with the protocol outlined in the IgM detection kit (Shanghai Enzyme-linked Biotechnology Co., Ltd., catalog number M1246L96).

Ethical approval

This study was approved by the Medical Ethics Committee of Huaibei Mining General Hospital in Huaibei City, Anhui Province, China (Approval No.2024 - 003). All subjects in the study were informed of all study items, including potential adverse effects, and signed an informed consent form.

STATISTICAL ANALYSIS

Utilizing SPSS 28.0 software, a statistical analysis was conducted. Values were presented as mean ± standard deviation ($\bar{x}\pm s$). The T-test was conducted to compare the lesion area and VAS scores, as well as to assess the differences in TNF-α, IL-17 and IL-6 levels before and after the treatment period. The Mann-Whitney U test was employed to evaluate the clinical efficacy between the study and control groups. The χ^2 test was applied to compare the occurrence of adverse reactions between the two groups. A statistical significance threshold was set at a P-value of <0.05.

Table 1: General data analysis

Group	*N*	Age (years)	Gender (male/female)	Duration (months)
Control	40	50.58±8.74	10/30	19.05±10.77
Study	40	50.45±8.71	16/24	18.93±10.19
t/χ^2		0.064	2.051	0.053
P		0.949	0.152	0.958

Table 2: Comparison of lesion area score and VAS score between the two groups

Group	*N*		lesion area score	VAS score
Control	40	Before treatment	3.96±0.78	7.81±0.84
		After treatment	3.21±0.83[a]	6.08±1.09[a]
Study	40	Before treatment	4.00±0.59	7.74±0.94
		After treatment	2.35±0.83[ab]	4.94±0.87[ab]

Note: a: Compared to the same group before treatment $P<0.05$; b: compared to the after treatment control group $P<0.05$.

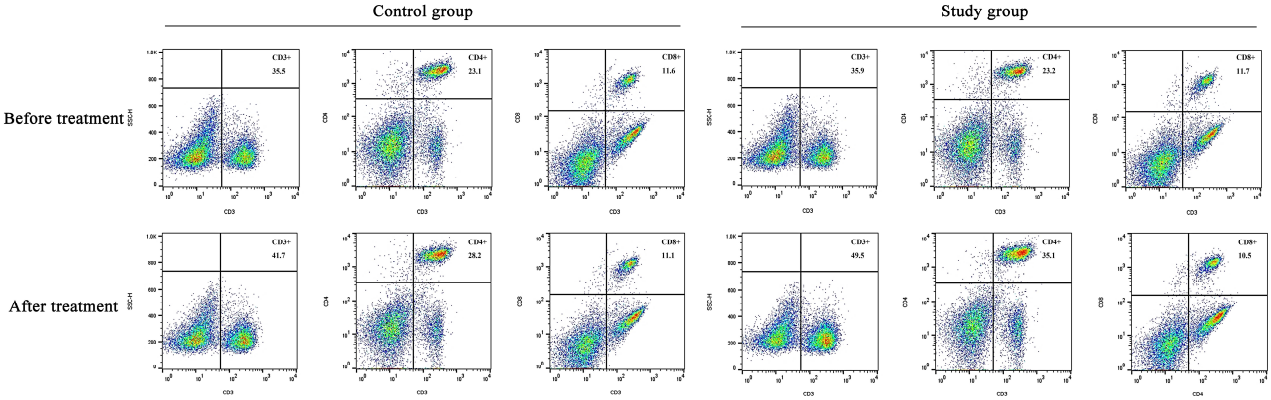

Fig. 1: Proportion of blood immune indexes: The proportion of CD3+, CD4+ and CD8+ T cells in the blood of the two groups was detected by flow cytometry.

RESULTS

General data analysis

In both the study and control groups, there were 40 cases each. table 1 indicated that there were no statistically significant differences in age, sex, or disease duration between the two groups ($P>0.05$). As evident from the data presented in table 1.

Comparison of objective index sign score and subjective symptom pain score

The results of the comparison of objective index sign scores and subjective symptom pain scores between the two groups of patients are expressed in table 2, there were no statistically significant variances in lesion area scores and VAS scores between the two groups before treatment ($P>0.05$). After treatment, both groups exhibited significantly reduced lesion area scores and VAS scores compared to their baseline measurements, with the study group showing significantly lower scores than the control group, and these differences were statistically significant ($P<0.05$).

Comparison of clinical efficacy

After treatment, the overall clinical effectiveness rate in the study group exceeded that of the control group ($P<0.05$). As evident from the data presented in table 3.

Changes in the expression of inflammatory factors

The results of changes in the expression of inflammatory factors before and after treatment are shown in table 4. There was no notable difference in the levels of TNF-α, IL-17 and IL-6 between the two groups before treatment ($P>0.05$). After treatment, the expression levels of salivary TNF-α, IL-17 and IL-6 in both groups significantly decreased, with the study group showing markedly lower levels than the control group ($P<0.05$).

Changes of blood immune indexes

The changes in blood immunity indexes before and after treatment are shown in table 5 and fig. 1. After treatment, the percentages of CD3+, CD4+ T cells and CD4+/CD8+ ratios in both groups showed a significant increase compared to their levels before treatment, while the proportion of CD8+ T cells exhibited no significant change ($P>0.05$). Following treatment, the percentages of CD3+, CD4+ T cells and CD4+/CD8+ ratios in the study group were greater than those in the control group, with the differences being statistically significant ($P<0.05$).

Analysis of safety indicators

The vital signs of both groups remained stable before and after treatment according to the results in table 6. During the treatment period, 1 patient in the study group

Table 3: Comparison of clinical efficacy between the two groups

Group	N	Excellent	Effective	Invalid	Total effective rate	*Z*	*P*
Control	40	10	12	18	55%	-2.583	0.01
Study	40	18	15	7	82.5%[a]		

Table 4: Changes in expression of inflammatory factors before and after treatment between the two groups

Group	N		TNF-α(ng/L)	IL-17(ng/L)	IL-6(ng/L)
Control	40	Before treatment	3.70±0.86	8.04±0.98	6.00±1.05
		After treatment	2.35±0.6[a]	6.95±0.89[a]	4.16±1.04[a]
Study	40	Before treatment	3.68±0.98	7.92±1.11	5.95±0.92
		After treatment	1.81±0.61[ab]	4.55±0.83[ab]	3.12±0.85[ab]

Note: a: Compared to the same group before treatment $P<0.05$; b: compared to the after treatment control group $P<0.05$.

Table 5: Changes of blood immune indexes

Group	N		CD3+(%)	CD4+(%)	CD8+(%)	CD4+/CD8+	IgM(g/L)
Control	40	Before treatment	35.54±3.36	23.07±2.94	11.67±1.65	2.01±0.38	1.74±0.60
		After treatment	41.73±3.01[a]	28.26±2.12[a]	11.15±1.39	2.57±0.37[a]	1.44±0.64[a]
Study	40	Before treatment	35.89±3.75	23.22±2.38	11.72±2.41	2.07±0.51	1.89±0.70
		After treatment	49.51±3.32[ab]	35.19±2.13[ab]	10.50±1.68	3.44±0.66[ab]	1.08±0.44[ab]

Note: a: Compared to the same group before treatment $P<0.05$; b: compared to the after treatment control group $P<0.05$.

Table 6: Comparison of the incidence of adverse reactions between the two groups during treatment

Group	N	Dizziness and headache	Nausea and vomiting	Elevated blood pressure	total	χ^2	*P*
Control	40	2	3	1	6(15%)	0.139	0.933
Study	40	1	2	1	4(10%)		

experienced dizziness and headaches, 2 patients had nausea and vomiting and 1 patient suffered from elevated blood pressure; the incidence of adverse reactions was 10% (4/40). In the control group, 2 patients reported dizziness and headaches, 3 patients experienced nausea and vomiting, and 1 patient had elevated blood pressure; the incidence of adverse reactions was 15% (6/40). No significant difference was found between the two groups ($\chi^2 = 0.139$, $P = 0.933$).

Recurrence comparison

During the 12-week follow-up period after treatment, the control group had 18 cases of recurrence, resulting in a recurrence rate of 45%, while the study group had 9 cases of recurrence, yielding a rate of 22.5%. The rate of recurrence in the study group was considerably less than that in the control group ($P<0.05$).

DISCUSSION

EOLP is a chronic non-communicable immune disease mediated by T cells (González-Moles *et al.*, 2021). According to statistics, the global prevalence rate is about 0.1% ~ 4%, ranking second in various oral mucosal diseases (Zhang *et al.*, 2022). Studies have shown that the abnormal immune response of oral mucosal epithelial cells mediated by T lymphocytes is one of the important pathogenesis of EOLP and the alterations in the oral microbial community and the inflammatory response also contribute significantly to the onset and progression of EOLP (Anitua *et al.*, 2022; Qing *et al.*, 2023). Nevertheless, the clinical treatment mainly focuses on immune response, micro ecological imbalance and inflammatory response, in order to alleviate the pain of the affected area, reduce the recurrence of the lesion, promote mucosal healing, control and reduce the risk of cancer as much as possible (Yang *et al.*, 2022). Clinically, corticosteroid hormone drugs are mainly used to treat oral lichen planus. These medications can partially suppress the production and progression of inflammatory mediators like tumor necrosis factor and interleukin, further regulate the release of these mediators, and contribute to the management of EOLP (Lodi *et al.*, 2020; Louisy *et al.*, 2024). However, these medications have a higher level of gastrointestinal irritation, which may lead to side effects like gastrointestinal disorders, insomnia, and blood pressure, and have a poor effect on EOLP recurrence control (Wu *et al.*, 2024).

Tacrolimus is a macrolide antibiotic belonging to a calcineurin inhibitor (Pinto *et al.*, 2023). It can effectively inhibit the calcium-dependent signaling pathway in T cells, thereby blocking the synthesis and transcription of cytokines in immune cells, and controlling the release of inflammatory mediators (Shilpa *et al.*, 2014; Utz *et al.*, 2022). Moreover, local administration of tacrolimus is

more convenient, which can directly act on the inflammatory area, penetrate the mucosal epithelium of the inflammatory response and then play a drug role (Guo *et al.*, 2015; Ribero *et al.*, 2015). Thomson *et al.* (2004) treated EOLP by topical application of tacrolimus ointment, and found that the erosion area decreased and the pain symptoms improved significantly after 6 weeks, which aligned with the findings of the control group in this research. Although local symptomatic treatment is an important means of treating EOLP patients, it can alleviate the progression of the patient's condition, but it needs long-term use (Rebora, 2017).

PRP gels contain a diverse range of growth factors. These bioactive substances can influence inflammation response, cell growth, stem cell movement, and blood vessel formation, thus enhancing repair and regeneration capabilities and speeding up lesion healing (ElGhareeb *et al.*, 2023; Sethi Ahuja *et al.*, 2020). Research has indicated that PRP may serve as a viable alternative for treating EOLP (Maddheshiya *et al.*, 2023). Hijazi *et al.* (2022) used the local injection of PRP lesions to treat OLP patients. The results showed that PRP lesion injection could effectively relieve subjective pain and objective clinical score to achieve better therapeutic effect. Research has demonstrated that PRP not only exerts a considerable impact in managing EOLP but is also safer than corticosteroids (Sethi Ahuja *et al.*, 2020). Therefore, we treated the patients in the study group by combining PRP gel and tacrolimus in order to obtain better clinical efficacy.

In this research, the clinical significance of combining PRP gel with tacrolimus for EOLP patients was evaluated by establishing a control group. The findings indicated that, in comparison to patients in the control group who received only tacrolimus ointment, those in the combined study group exhibited notable benefits in overall effectiveness. Saglam *et al.* (2021) administered platelet-rich fibrin and methylprednisolone acetate to patients with bilateral EOLP. After treatment results revealed a marked decrease in lesion size scores and VAS scores for both groups, with no substantial differences between them. Similarly, Bennardo *et al.* (2021) and Al-Hallak *et al.* (2023) concluded that platelet-rich fibrin can effectively alleviate the severity of OLP lesions and associated pain. In this study, both the lesion area scores and VAS scores were markedly decreased, with the study group showing superior results compared to the control group. This may be attributed to the enhanced efficacy of the drug combination in alleviating pain compared to a single agent.

OLP is a persistent inflammatory and autoimmune condition. The pathological alterations in the mucosal tissues associated with OLP may result in modifications to the oral immune microenvironment (Popovska *et al.*, 2013). The progression of this autoimmune condition is mainly influenced by T cells, which secrete inflammatory cytokines such as IL-6, IL-8, IL-17 and TNF-*α* (Lu *et al.*, 2015). Mozaffari *et al.* (2018) discovered that levels of IL-6 in saliva were markedly elevated in patients with OLP compared to individuals without the condition. Furthermore, several investigations have noted a notable rise in IL-17 expression in both serum and saliva of oral lichen planus patients, showing a positive correlation with disease severity scores (Afzali *et al.*, 2023; K. Wang *et al.*, 2015). TNF-*α* is linked to the pathogenesis of autoimmune conditions such as discoid lupus erythematosus, pemphigus, Behcet's disease, and OLP. It serves as a crucial pro-inflammatory mediator that can influence the production and apoptosis of other cytokines (DeAngelis *et al.*, 2023). The results of this research indicated that the concentrations of TNF-*α*, IL-17, and IL-6 in the saliva of both groups after four weeks of therapy were reduced compared to levels before treatment, with the study group showing lower levels than the control group after treatment. Jiang *et al.* (2024) reported consistent findings by analyzing changes in salivary levels of inflammatory cytokines in a study of the glucocorticosteroid prednisone acetate in the treatment of patients with vesicular oral lichen planus. This suggests that the drug combination can more effectively reduce the expression of inflammatory cytokines.

Research has indicated that the underlying cause of OLP may involve an imbalance in T lymphocyte subsets. In the context of the body's immune response, T lymphocyte subsets are responsible for cellular immunity function based on the relative makeup of CD3+, CD4+ and CD8+. The CD4+/CD8+ ratio can directly indicate cellular immune status and the equilibrium between these subsets (Rassol and Zaidan, 2023; H. Wang *et al.*, 2016). Findings suggest that T lymphocyte subsets in patients with EOLP are notably characterized by a marked reduction in CD3+, CD4+ T cells, and the CD4+/CD8+ ratio, alongside an increase in IgM expression (Maehara *et al.*, 2015). This research revealed that the levels of CD3+, CD4+ and CD4+/CD8+ in both groups were notably increased compared to pre-treatment levels, while CD8+ and IgM levels were significantly reduced. The study group exhibited higher levels of CD3+, CD4+ and CD4+/CD8+ than the control group, while CD8+ and IgM levels were lower in the study group compared to the control group. Chen *et al.* (2024) reached consistent conclusions by analyzing T-cell changes in patients with erosive oral lichen planus treated with corticosteroids. These findings suggest that the use of PRP gel along with tacrolimus ointment effectively rebalances the cellular immune markers and T lymphocyte subgroups within the study cohort.

Safety concerns during patient treatment are usually analyzed through side effects or adverse reaction profiles

during treatment (Singh *et al.*, 2022). The results of the study showed that there was no significant difference between the incidence of adverse reactions such as dizziness and headache, nausea and vomiting, and elevated blood pressure between the two groups of patients. In addition, the relapse rate of patients in the study group was significantly lower than that of the control group during the 12-week follow-up period. These results suggest that the safety profiles of treatment with tacrolimus ointment alone and tacrolimus ointment in combination with PRP gel are similar and that patients on the combination had a lower relapse rate within 12 weeks.

CONCLUSION

In summary, PRP gel combined with tacrolimus ointment and single use of tacrolimus ointment can effectively treat EOLP, but the combined treatment effect is better, which can effectively improve clinical symptoms, reduce lesion area, relieve pain, and have fewer adverse reactions. This could be linked to the decrease in inflammatory mediators and the facilitation of immune balance restoration. This approach offers novel therapeutic concepts for patients with underlying conditions and challenging EOLP cases. Additionally, PRP gel may demonstrate significant clinical efficacy in severe EOLP patients unable to tolerate steroids, warranting further clinical promotion and extensive research. Due to differences in individual dietary lifestyle habits, a 12-week follow-up period may not be sufficient to assess long-term outcomes and potential delayed side effects, which could be further analyzed for long-term efficacy and assessed for safety in future studies.

REFERENCES

Afzali S, Mohammad isoleimani E, Mansoori Y, Mohaghegh P, Bahmanyar M, Mansoori B, Pezeshki B, Nikfar G, Tavassoli A, Shahi A and Moravej A (2023). The potential roles of Th17 cells in the pathogenesis of oral lichen planus. *Inflamm Res.,* **72**(7): 1513-1524.

Al-Hallak N, Hamadah O, Mouhamad M and Kujan O (2023). Efficacy of injectable platelet-rich fibrin in the treatment of symptomatic oral lichen planus. *Oral Dis.,* **29**(5): 2256-2264.

Alarcon-Sanchez M A, Becerra-Ruiz J S, Avetisyan A and Heboyan A (2024). Activity and levels of TNF-α, IL-6 and IL-8 in saliva of children and young adults with dental caries: A systematic review and meta-analysis. *BMC Oral Health,* **24**(1): 816.

Almutairi M, Riyaz SSMA, Awinashe M and Almutairi FJ (2023). Assessment of 5% amlexanox, 0.1% triamcinolone acetonide and 0.03% tacrolimus in the management of oral lichen planus. *J. Pharm. Bioallied Sci.,* **15**(Suppl 2): S1298-S1300.

Anitua E, Eguia A, Alkhraisat MH and Piñas L (2022). Oral lichen planus treated with plasma rich in growth factors. *Cutis,* **109**(3): 163-166.

Arana A, Pottegård A, Kuiper J G, Booth H, Reutfors J, Calingaert B, Lund L C, Crellin E, Schmitt-Egenolf M, Kaye J A, Gembert K, Rothman K J, Kieler H, Dedman D, Houben E, Gutiérrez L, Hallas J and Perez-Gutthann S (2021). Long-term risk of skin cancer and lymphoma in users of topical tacrolimus and pimecrolimus: Final results from the extension of the cohort study protopic joint european longitudinal lymphoma and skin cancer evaluation (JOELLE). *Clin. Epidemiol.,* **13**: 1141-1153.

Bennardo F, Liborio F, Barone S, Antonelli A, Buffone C, Fortunato L and Giudice A (2021). Efficacy of platelet-rich fibrin compared with triamcinolone acetonide as injective therapy in the treatment of symptomatic oral lichen planus: A pilot study. *Clin. Oral. Investig.,* **25**(6): 3747-3755.

Chen S, Wu X, Yang Y, Xu X, Xiong X and Meng W (2024). Increased pathogenicity and pro-inflammatory capabilities of mucosal-associated invariant T cells involved in oral lichen planus. *BMC Oral Health,* **24**(1): 829.

DeAngelis LM, Cirillo N, Perez-Gonzalez A and McCullough M (2023). Characterization of mucosal-associated invariant t cells in oral lichen planus. *Int. J. Mol. Sci.,* **24**(2): 1490.

Didona D, Caposiena Caro R D, Sequeira Santos A M, Solimani F and Hertl M (2022). Therapeutic strategies for oral lichen planus: State of the art and new insights. *Front Med (Lausanne),* **9**: 997190.

Djukic T, Drvenica I, Kovacic M, Milanovic S, Majeric D, Sefik-Bukilica M, Miletic M, Bugarski B and Ilić V (2024). Exploring the link between hydrodynamic size and immunoglobulins of circulating immune complexes in rheumatoid arthritis patients. *Int. J. Mol. Sci.,* **25**(6): 3138.

Elenbaas A, Enciso R and Al-Eryani K (2022). Oral lichen planus: A review of clinical features, etiologies, and treatments. *Dent. Rev.,* **2**(1): 100007.

ElGhareeb MI, Ghoneimy S and Elsayed A (2023). Intralesional injection of platelet-rich plasma versus steroid in the treatment of oral lichen planus. *J Cosmet. Dermatol.,* **22**(5): 1481-1487.

Godoi TTF, Rodrigues BL, Huber SC, Santana MHA, da Fonseca LF, Santos GS, Azzini GOM, Mosaner T, Paulus-Romero C and Lana JFSD (2022). Platelet-rich plasma gel matrix (PRP-GM): Description of a new technique. *Bioengineering,* **9**(12): 817.

Gonzalez-Moles M, Warnakulasuriya S, Gonzalez-Ruiz I, Gonzalez-Ruiz L, Ayen A, Lenouvel D, Ruiz-Ávila I and Ramos-García P (2021). Worldwide prevalence of oral lichen planus: A systematic review and meta-analysis. *Oral Dis.,* **27**(4): 813-828.

Guo CL, Zhao JZ, Zhang J and Dong HT (2015). Efficacy of topical tacrolimus for erosive oral lichen planus: A meta-analysis. *Chin. Med. Sci. J.,* **30**(4): 210-217.

Gururaj N, Hasinidevi P, Janani V and Divynadaniel T (2021). Diagnosis and management of oral lichen planus – Review. *J. Oral Maxillofac. Pathol.,* **25**(3): 383-393.

Hettiarachchi P, Hettiarachchi RM, Jayasinghe RD and Sitheeque M (2017). Comparison of topical tacrolimus and clobetasol in the management of symptomatic oral lichen planus: A double-blinded, randomized clinical trial in Sri Lanka. *J. Investig. Clin. Dent.,* **8**(4): 12237.

Hijazi A, Ahmed W and Gaafar S (2022). Efficacy of intralesional injections of platelet-rich plasma in patients with oral lichen planus: A pilot randomized clinical trial. *Clin. Exp. Dent Res.,* **8**(3): 707-714.

Huber SC, de Lima Montalvao S A, Sachetto Z, Santos Duarte Lana JF and Annichino-Bizzacchi JM (2021). Characterization of autologous platelet rich plasma (PRP) and its biological effects in patients with Behcet's disease. *Regen. Ther.,* **18**: 339-346.

Ismail F F and Sinclair R (2020). Clinical healing of erosive oral lichen planus with tildrakizumab implicates the interleukin-23/interleukin-17 pathway in the pathogenesis of lichen planus. *Australas J. Dermatol.,* **61**(2): e244-e245.

Jain N K and Gulati M (2016). Platelet-rich plasma: a healing virtuoso. *Blood Res.,* **51**(1): 3-5.

Jiang L, Huang Y, Fang M, Chen X, Feng D, Liu J, Jiang Q and Tao R (2024). Dynamic changes of Th1/Th2/Th17 cytokines and hBD-2/3 in erosive oral lichen planus patients saliva before and after prednisone acetate treatment. *Heliyon,* **10**(1): e24043.

Kia S J, Basirat M, Mortezaie T and Moosavi M S (2020). Comparison of oral nano-curcumin with oral prednisolone on oral lichen planus: A randomized double-blinded clinical trial. *BMC Complement. Med. Ther.,* **20**(1): 328.

Li C, Tang X, Zheng X, Ge S, Wen H, Lin X, Chen Z and Lu L (2020). Global prevalence and incidence estimates of oral lichen planus: A systematic review and meta-analysis. *JAMA Dermatol.,* **156**(2): 172-181.

Lodi G, Manfredi M, Mercadante V, Murphy R and Carrozzo M (2020). Interventions for treating oral lichen planus: Corticosteroid therapies. *Cochrane Database Syst. Rev.,* **2**(2): Cd001168.

Louisy A, Humbert E and Samimi M (2024). Oral lichen planus: An update on diagnosis and management. *Am. J. Clin. Dermatol.,* **25**(1): 35-53.

Lu R, Zhang J, Sun W, Du G and Zhou G (2015). Inflammation-related cytokines in oral lichen planus: An overview. *J. Oral Pathol. Med.,* **44**(1): 1-14.

Ma W, Wei S, Long S, Tian E C, McLaughlin B, Jaimes M, Montoya D J, Viswanath V R, Chien J and Zhang Q (2023). Dynamic evaluation of blood immune cells predictive of response to immune checkpoint inhibitors in NSCLC by multicolor spectrum flow cytometry. *Front. Immunol.,* **14**: 1206631.

Maddheshiya N, Srivastava A, Rastogi V, Shekhar A, Sah N and Kumar A (2023). Platelet-rich plasma protein as a therapeutic regimen for oral lichen planus: An evidence-based systematic review. *Natl. J. Maxillofac Surg,* **14**(1): 22-26.

Maehara T, Moriyama M, Kawano S, Hayashida J N, Furukawa S, Ohta M, Tanaka A, Yamauchi M, Ohyama Y, Kiyoshima T and Nakamura S (2015). Cytokine profiles contribute to understanding the pathogenic difference between good syndrome and oral lichen planus: Two case reports and literature review. *Medicine (Baltimore),* **94**(14): e704.

Merigo E, Oppici A, Parlatore A, Cella L, Clini F, Fontana M and Fornaini C (2018). Platelet-rich plasma (PRP) rinses for the treatment of non-responding oral lichen planus: a case report. *Biomedicines,* **6**(1): 15.

Meznerics F A, Fehervari P, Dembrovszky F, Kovacs K D, Kemény L V, Csupor D, Hegyi P and Banvölgyi A (2022). Platelet-rich plasma in chronic wound management: A systematic review and meta-analysis of randomized clinical trials. *J. Clin. Med.,* **11**(24): 7532.

Mozaffari H R, Sharifi R and Sadeghi M (2018). Interleukin-6 levels in the serum and saliva of patients with oral lichen planus compared with healthy controls: A meta-analysis study. *Cent. Eur. J. Immunol.,* **43**(1): 103-108.

Ozdemir S, Parlakyıldız Gokce A and Unver T (2020). Simulation of three intraoral radiographic techniques in pediatric dental patients: Subjective comfort assessment using the VAS and Wong-Baker FACES Pain Raiting Scale. *BMC Oral Health,* **20**: 1-7.

Pinto J, Waghmare M, Bhor K, Santosh V, Manoj R and Samson S (2023). Efficacy and safety of topical tacrolimus in comparison with topical corticosteroids, calcineurin inhibitors, retinoids and placebo in oral lichen planus: An updated systematic review and meta-analysis. *Asian Pac. J. Cancer Prev.,* **24**(2): 389-400.

Popovska M, Grchevska L, Popovski V, Atanasovska-Stojanovska A, Minovska A, Belazelkoska Z, Radojkova-Nikolovska V, Mitik K and Strezovska S (2013). T-cell subpopulations in lesions of oral lichen planus. *Pril (Makedon Akad Nauk Umet Odd Med Nauki),* **34**(2): 143-149.

Qing M, Yang D, Shang Q, Peng J, Deng J, Lu J, Li J, Dan H, Zhou Y, Xu H and Chen Q (2023). $CD8^+$ tissue-resident memory T cells induce oral lichen planus erosion via cytokine network. *Elife,* **12**: e83981.

Rassol HJ and Zaidan TF (2023). Immunohistochemical expression of T-cell subsets (CD4 and CD8) in oral lichen planus. *Pan. Afr. Med. J.,* **45**: 147.

Rebora A (2017). Tacrolimus and oral lichen planus: The question remains. *J. Eur. Acad. Dermatol. Venereol.,* **31**(2): e86.

Ribero S, Stieger M, Quaglino P, Hongang T, Bornstein M M, Naldi L and Borradori L (2015). Efficacy of topical tacrolimus for oral lichen planus: Real-life experience in a retrospective cohort of patients with a review of the literature. *J. Eur. Acad. Dermatol. Venereol.,* **29**(6): 1107-1113.

Rotaru D I, Sofineti D, Bolboaca S D and Bulboacă A E (2020). Diagnostic criteria of oral lichen planus: A narrative review. *Acta Clin. Croat.,* **59**(3): 513-522.

Saglam E, Ozsagir Z B, Unver T, Alinca S B, Toprak A and Tunali M (2021). Efficacy of injectable platelet-rich fibrin in the erosive oral lichen planus: A split-mouth, randomized, controlled clinical trial. *J. Appl. Oral Sci.,* **29**: e20210180.

Sethi Ahuja U, Puri N, More C B, Gupta R and Gupta D (2020). Comparative evaluation of effectiveness of autologous platelet rich plasma and intralesional corticosteroids in the management of erosive oral Lichen planus - a clinical study. *J. Oral Biol. Craniofac. Res.,* **10**(4): 714-718.

Shilpa PS, Kaul R, Bhat S, Sanjay CJ and Sultana N (2014). Topical tacrolimus in the management of oral lichen planus: Literature review. *J. Calif. Dent. Assoc.,* **42**(3): 165-170.

Singh A, Khillan R, Mishra Y and Khurana S (2022). The safety profile of COVID-19 vaccinations in the United States. *Am. J. Infect. Control,* **50**(1): 15-19.

Smith O J, Talaat S, Tomouk T, Jell G and Mosahebi A (2022). An evaluation of the effect of activation methods on the release of growth factors from platelet-rich plasma. *Plast. Reconstr. Surg,* **149**(2): 404-411.

Sridharan K and Sivaramakrishnan G (2021). Interventions for oral lichen planus: A systematic review and network meta-analysis of randomized clinical trials. *Aust. Dent. J.,* **66**(3): 295-303.

Sriram S, Hasan S, Alqarni A, Alam T, Kaleem S M, Aziz S, Durrani H K, Ajmal M, Dawasaz A A and Saeed S (2023). Efficacy of platelet-rich plasma therapy in oral lichen planus: A systematic review. *Medicina (Kaunas),* **59**(4): 746.

Su Z, Hu J, Cheng B and Tao X (2022). Efficacy and safety of topical administration of tacrolimus in oral lichen planus: An updated systematic review and meta-analysis of randomized controlled trials. *J. Oral Pathol. Med.,* **51**(1): 63-73.

Sun SL, Liu JJ, Zhong B, Wang JK, Jin X, Xu H, Yin FY, Liu T N, Chen Q M and Zeng X (2019). Topical calcineurin inhibitors in the treatment of oral lichen planus: A systematic review and meta-analysis. *Br. J. Dermatol.,* **181**(6): 1166-1176.

Thomson MA, Hamburger J, Stewart DG and Lewis HM (2004). Treatment of erosive oral lichen planus with topical tacrolimus. *J Dermatolog Treat.,* **15**(5): 308-314.

Utz S, Suter V G A, Cazzaniga S, Borradori L and Feldmeyer L (2022). Outcome and long-term treatment protocol for topical tacrolimus in oral lichen planus. *J. Eur. Acad. Dermatol. Venereol.,* **36**(12): 2459-2465.

Wang H, Zhang D, Han Q, Zhao X, Zeng X, Xu Y, Sun Z and Chen Q (2016). Role of distinct $CD4^+$ T helper subset in pathogenesis of oral lichen planus. *J. Oral Pathol. Med.,* **45**(6): 385-393.

Wang K, Miao T, Lu W, He J, Cui B, Li J, Li Y and Xiao L (2015). Analysis of oral microbial community and Th17-associated cytokines in saliva of patients with oral lichen planus. *Microbiol. Immunol.,* **59**(3): 105-113.

Wu T, Bai Y, Jing Y and Chen F (2024). What can we learn from treatments of oral lichen planus? *Front. Cell Infect. Microbiol.,* **14**: 1279220.

Yang J Y, Tan Y Q and Zhou G (2022). T cell-derived exosomes containing cytokines induced keratinocytes apoptosis in oral lichen planus. *Oral Dis.,* **28**(3): 682-690.

Zhang Y, Liu K, Cheng J, Zhou C, Zhang M and Fan Y (2022). FAP-α^+ immunofibroblasts in oral lichen planus promote $CD4^+$ T-cell infiltration via CCL5 secretion. *Exp. Dermatol.,* **31**(9): 1421-1430.