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Efficacy of Combined vs. Monotherapy in Oral Lichen Planus: A Randomized Clinical Trial

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ABSTRACT

Objectives: The aim of this study was to compare the topical therapeutic efficacy of triamcinolone acetonide (TA) (0.2%) plus hyaluronic acid (HA) (0.1%) versus monotherapy in patients with symptomatic oral lichen planus (OLP), as well as to investigate the oxidative stress of saliva under the different treatments.

Materials and Methods: Sixty OLP patients were included in a randomized, double-blind, singlecenter study with a treatment duration of 28 days and 3-month follow-up period. Participants were randomized into three groups: Group I (TA + HA), Group II (TA), and Group III (HA). Treatment efficacy was assessed by means of Thongprasom scale, Oral Health Impact Profile-14 (OHIP-14), and visual analog scale (VAS). In addition, biochemical analyses were performed in order to determine the level of antioxidant biomarkers in saliva, including superoxide dismutase (SOD), glutathione (GSH), and total antioxidant capacity (TAC).

Results: All treatments seem to exhibit a significant effect in accordance with Thongprasom scale ($p < 0.001$), VAS reduction ($p < 0.001$), and OHIP14 ($p < 0.05$), which maintains over time. No significant changes in salivary oxidative stress in any of the three groups occurred.

Conclusions: The results exhibited a significant improvement in the treated patients in all three groups. There were no significant changes in salivary stress biomarkers under treatment condition.

1 | Introduction

Lichen planus is a severe mucocutaneous inflammatory disease characterized by its autoimmune nature, affecting both the skin and mucous membranes. Its etiopathogenesis remains unknown (Louisy, Humbert, and Samimi 2024; Warnakulasuriya et al. 2021; Alrashdan, Cirillo, and McCullough 2016). Oral lichen planus (OLP) presents a wide spectrum of clinical manifestations. Over time, the lesions may undergo periods of exacerbation or remission. Its different clinical presentations can be grouped into two categories: white-keratotic lesions (reticular, papular, and plaque-like form) and red-erosive lesions (atrophic, erosive/ulcerative, and vesicular form) (Louisy, Humbert, and Samimi 2024). The World Health Organization (WHO) considers OLP as an oral potentially malignant disorder (OPMD). Therefore, it is essential to control and treat OLP lesions (Warnakulasuriya et al. 2021). Several treatment regimens have been designed for OLP: corticosteroids, cyclosporine, tacrolimus, pimecrolimus, retinoids and vitamin A analogues, hydroxychloroquine, dapsone, griseofulvin, methotrexate;

azathioprine among others. The main problem with current therapies used for OLP is their side effects, which include infectious problems, burning sensation, stinging, dysgeusia and immunosuppression, hepatotoxicity and anemia among others (Garcia-Pola, Gonzalez-Alvarez, and Garcia-Martin 2017; Polizzi et al. 2023; Santonocito et al. 2020; Wu et al. 2024; Lodi et al. 2020). These side effects occur especially when the treatments are used for the long-term management of chronic diseases.

Oxidative stress has been implicated in various chronic inflammatory diseases, including OLP (Battino et al. 2008; Darczuk et al. 2016). Previous studies have shown that patients with OLP exhibit elevated levels of reactive oxygen species and a decreased antioxidant capacity in saliva. Evaluating antioxidant biomarkers in saliva allows us to better understand the relationship between oxidative stress and the severity of symptoms in OLP, and how treatments may influence this balance (Tvarijonaviciute et al. 2017).

Among the various treatments, corticosteroids like TA (triamcinolone acetonide), despite their side effects are the first-line treatment for OLP due to their powerful anti-inflammatory effects; safety and cost (Wu et al. 2024; Lodi et al. 2020). They help reduce symptoms, such as pain, redness and inflammation of mucosal lesions. Also, hyaluronic acid is useful for OLP due to its healing properties, ability to improve hydration and the integrity of the mucosal barrier and lack of side effects (Waingade, Medikeri, and Gaikwad 2022; Shetty, Burde, and Guttal 2016; Hashem et al. 2019; Nolan et al. 2006). Although HA shows good activities properties, data on its use in OLP are scanty and contradictory. This is because the existing studies on HA present numerous biases, such as sample size, study design, methodology, and measurement of results (Waingade, Medikeri, and Gaikwad 2022; Shetty, Burde, and Guttal 2016; Hashem et al. 2019; Nolan et al. 2006; Casale et al. 2016).

Shetty, Burde, and Guttal (2016) in his study of patients with OLP, he grouped them into two categories. One group was given HA while the other group was placed on placebo. The group treated with HA had reduced symptoms, including burning sensation, erythema, and erosions relative to the group treated with placebo. The finding shows that HA (hyaluronic acid) stimulates angiogenesis, reduces exudation, is vasoprotective, and exerts fibrogenic action in inflamed tissues with altered healing.

Here, we analyzed the combination impact of TA and HA on OLP. Both agents allow an interesting approach. While TA controls the immune response and reduce inflammation, hyaluronic acid improves repair and protection (Wu et al. 2024; Lodi et al. 2020; Waingade, Medikeri, and Gaikwad 2022) possibly reducing the need for high doses of TA, limiting their side effects. The importance of this study is to offer an alternative to conventional treatments for OLP, particularly in cases where prolonged steroid use may be disturbing.

The major aim of this work was to evaluate whether the combination of triamcinolone acetonide (0.2%) and hyaluronic acid (0.1%) is more effective than monotherapy (either TA or HA) in decreasing the severity of symptoms and enhancing the participants' life quality with symptomatic OLP. It also investigates the impacts of the treatments on the antioxidant status of saliva.

2 | Material and Methods

2.1 | Study Design

This study design is a double-blind, randomized controlled trial carried out over 4 weeks with a 3-month follow-up after the treatment plan was completed. Approval was obtained from the local ethical committee prior to the study (ID: 3938/2022). Written informed consent was given by the study participants. The research was conducted in accordance with the ethical rules established in the Declaration of Helsinki of the World Medical Association. It was registered at clinicaltrials.gov (NCT06332365) and conducted according to the CONSORT 2010 guidelines.

To ensure anonymity and impartiality, a computerized randomization list was generated by an independent person. The treatments, allocated by an external pharmacist not involved in the protocol, were packaged in identical bottles numbered according to randomization. Each sealed envelope contained the protocol assignment for individual patients and written instructions for the application of the medication. Both investigators and participants remained blinded to treatment assignment until the end of the statistical analysis. The subjects were grouped into 3:

- Group I: Treated with triamcinolone acetonide 0.2% + hyaluronic acid 0.1% in orabase;
- Group II: Treated with triamcinolone acetonide 0.2% in orabase; and
- Group III: Treated with hyaluronic acid 0.1% in orabase.

During the treatment period, neither the researcher nor the subjects knew which treatments were being used.

2.2 | Study Participants

Oral Medicine patients were consecutively recruited from the University Dental Clinic of the Morales Meseguer Hospital. The inclusion criteria were participants must be over 18 years of age, participants must meet the clinical and histological diagnostic criteria for OLP, according to Van der Meij and van der Waal (2003), (1) presence of bilateral symmetric lesions; (2) presence of lace-like network of slightly raised gray-white lines (reticular or linear pattern), with atrophies or erosions or ulcerations; (3) with mucosal biopsies confirmed with OLP and they must present painful lesions and sign the consent form. Exclusion criteria included: under 18 years of age, the appearance of dysplasia in the histopathological sample, use of medications that induce lichenoid reactions (Antihypertensives such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers; nonsteroidal anti-inflammatory drugs); sulfonylureas; antimalarial medications; antiepileptic medications; gold salts and other medications used in the treatment of autoimmune diseases. Presence of amalgam fillings near the lesions,

having treated OLP in the past 6 weeks; pregnant women with hypersensitivity to any of the products used.

Once all patients included agreed to participate, a baseline saliva sample (T0) was taken and a supragingival oral cleaning was subsequently performed. It was carried out carefully to avoid disrupting the tissue, with the aim that everyone entered under the same conditions. Afterward, the indications and instructions for oral hygiene are given. They are not to use any mouthwash product different from that of the study. Product application measures are given below:

- Triamcinolone acetonide 0.2% + hyaluronic acid 0.1% in orabase; administered twice a day for 4 weeks.
- Triamcinolone acetonide 0.2% in orabase; administered twice a day for 4 weeks.
- Hyaluronic acid 0.1% in orabase; administered twice a day for 4 weeks.

The participants had to apply with their finger a sufficient amount of the treatment to cover the affected area thinly and evenly without exceeding the size of a pea, however, those with multiple painful areas were instructed to apply it to each area individually, which could result in a higher total amount of medication used. They were without food, water, and made not to talk for at least half an hour.

2.3 | Variables

Patients were evaluated by a detailed collection of anamnesis data and oral signs. Next, their mouths were examined conventionally with lesions observed and recorded by a single observer (RRG). Medical data were given based on the Thongprasom scoring system (0–5) (Thongprasom et al. 1992) used to grade the severity of OLP in relation to the existence and degree of white streaks, erythema, and atrophy. Score 0 points = no injury; normal mucosa; Score 1: hyperkeratotic lesions; Score 2: atrophic area $\leq 1\,cm^2$; Score 3: atrophic area $> 1\,cm^2$; Score 4: erosive area $\leq 1\,cm^2$; Score 5: erosive area $> 1\,cm^2$.

Subjective painful symptoms were measured using a visual analog scale (VAS). It evaluates the symptoms of OLP. It consists of 10 cm in length; 0 means absence of pain and 10 are extreme symptoms. To measure the severity of the lesions that presented desquamative gingivitis, the Arduino scale was used (Arduino et al. 2017). The scale includes a value for each sextant in the vestibular and another value in the lingual and palatal (12 in total, 6 in the vestibular, 3 in the lingual, and 3 palatine). When there are diverse features, the clinician must select the highest index, thus the overall score can differ from 0 to 48. The higher the score, the more chronic it is. The deterioration in quality of life was determined by the Oral Health Impact Profile (OHIP-14) (Montero-Martín et al. 2009). It consists of 7 subscales (the total score ranges between 0 and 56).

The Hospital anxiety-depression scale (HAD) was comprehensively used to analyze the patient's mental health. It consists of 14 questions that are scored from 0 to 3. The HAD scale has a maximum overall score of 21 for each subscale (anxiety and depression). However, a score of 8–10 is used as a clinical cutoff point to detect significant levels of anxiety or depression (Zigmond and Snaith 1983).

2.4 | Saliva Collection and Analysis

Unstimulated saliva was collected from the patients. Salivary fluid was collected over a period of 5 min according to the technique described by Navazesh and Kumar (2008). Samples were collected between 9:00 and 11:00 in the morning when participants did not eat, drink or brush their teeth. The saliva was centrifuged at 3000 *g* for 10 min, transferred to Eppendorf vials and stored at −80° until analyzed. Three samples were taken for each patient at different study times: At the inclusion of the study (T0), after supragingival hygiene (T1), and once the treatment was completed (T2). Samples contaminated with blood (using visual inspection scale) were discarded. Antioxidant capacity was measured using TEAC, FRAP, and CUPRAC, and enzymatic activity was determined by commercial SOD and GSH assays.

Saliva antioxidant capacity was measured in terms of Trolox equivalent antioxidant capacity (TEAC) (Erel 2004), plasma iron reducing ability (FRAP) (Benzie and Strain 1996) and copper reducing antioxidant capacity (CUPRAC) (Campos et al. 2009). Enzymatic antioxidant property was calculated as superoxide dismutase (SOD) activity and commercial assays were used to measure GSH according the rules of the manufacturer: Sigma-Aldrich, St. Louis, MO, USA.

2.5 | Study Plan

2.5.1 | Visit 1 (Day 1)

Inclusion Criteria: Clinical examination, confirmation of eligibility to participate in the study. Initial Procedures: Acceptance of the patient and collection of a global saliva sample at rest. A complete oral examination was performed. Evaluations: Record of the Thongprasom scale, visual analog scale (VAS), Oral Health Index (OHIP-14), Hospital Anxiety and Depression Scale (HAD), and measurement of desquamative gingivitis (Arduino et al. 2017). Interventions: Professional supragingival oral cleaning followed by personalized oral hygiene instructions. Second Sample: Taking a second global saliva sample. Treatment Assignment: Patients were assigned to treatment groups as appropriate.

2.5.2 | Visit 2 (Day 15)

Pain Assessment: Assessment of pain using the visual analog scale.

2.5.3 | Visit 3 (End of Treatment After 4 weeks)

Follow-up: Clinical examination and periodic evaluations with the Thongprasom scale, VAS, OHIP-14, desquamative gingivitis test score, and HAD. Saliva Sample: Collection of a global saliva sample. Compliance and Satisfaction Evaluation: Assessment of patients' compliance and satisfaction with treatment. All participants were instructed to bring the gel tubes to the 1-month

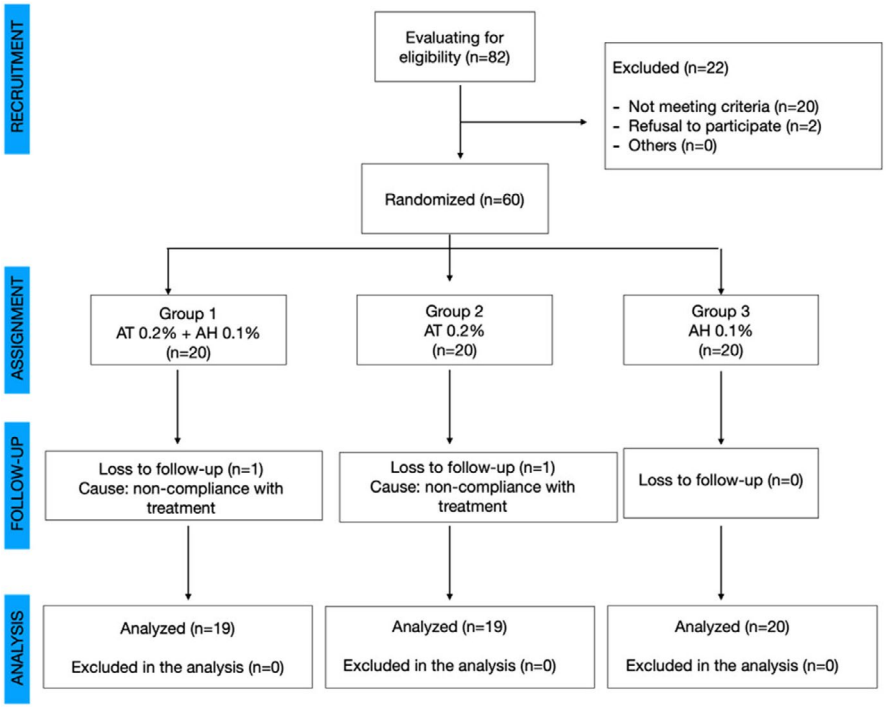

FIGURE 1 | Diagram flow.

TABLE 1 | Descriptive and comparative study of the variables between the different treatment groups.

	Group				
	TA + HA	**TA**	**HA**	**Test**	***p***
Gender					
Male	4 (20)	5 (25)	4 (20)	$\chi^2(1) = 0.196$	0.906
Female	16 (80)	15 (75)	16 (80)		
Age	63.6 (14.56)	62.25 (7.28)	62.55 (13.28)	$F(2;59) = 0.068$	0.934
Smoking					
No	9 (45)	12 (60)	15 (75)	$\chi^2(6) = 6.947$	0.326
Ex-smoker	9 (45)	7 (35)	3 (15)		
< 10	2 (10)	1 (5)	1 (5)		
> 10	0 (0)	0 (0)	1 (5)		
Alcohol					
No	11 (55)	10 (50)	13 (65)	$\chi^2(6) = 4.02$	0.674
1 glass/week	0 (0)	0 (0)	1 (5)		
2–3 glasses/week	8 (40)	9 (45)	6 (30)		
> 3 glasses/week	1 (5)	1 (5)	0 (0)		

Abbreviations: HA, hyaluronic acid; TA, triamcinolone acetonide.

follow-up appointment to measure the remaining weight of the gel with a calibrated scale.

2.5.4 | Visit 4 (3 Months After Completion of Treatment)

Pain Assessment: Assessment of pain using the visual analog scale (VAS) was conducted by phone.

2.6 | Statistical Analysis

The sample size was based on previous studies of our group. An alpha risk of 0.05 (95% confidence) and a beta risk of 0.2 (power 0.8) were accepted in a bilateral contrast for the comparison of means between the three groups. An independent samples *t*-test, assuming unknown, but equal variances was used to determine a high magnitude effect

TABLE 2 | Mean values (standard deviation SD) and statistical contrasts between treatments of the THONGPRASOM; test Arduino; OHIP-14; anxiety and depression.

	Time		Within-subject effects[a]			
	To start	Treatment completion in 4 Weeks	Time		Treatment × time	
	Mean (SD)	Mean (SD)	$F(1;55)$	p value (η^2)	$F(2;55)$	p value (η^2)
Thongprasom						
TA + HA	3.63 (1.61)	3.74 (1.42)	85.648	**< 0.001** (0.609)	0.705	0.499 (0.025)
TA	3.84 (1.39)	2.37 (1.26)				
HA	3.75 (1.33)	2.89 (1.37)				
Total	3.74 (1.42)	2.75 (1.48)				
Arduino						
TA + HA	5.26 (6.45)	4.98 (6.72)	16.573	**< 0.001** (0.232)	0.678	0.512 (0.024)
TA	4.68 (7.45)	3.00 (3.89)				
HA	5.00 (6.57)	3.53 (5.87)				
Total	4.98 (6.72)	3.55 (5.06)				
OHIP-14						
TA + HA	14.00 (7.12)	9.05 (6.00)	43	**< 0.001** (0.439)	2.22	0.118 (0.075)
TA	14.89 (6.72)	11.79 (9.07)				
HA	13.15 (8.57)	10.85 (8.42)				
Total	14.00 (7.43)	10.57 (7.89)				
Anxiety						
TA + HA	6.26 (3.84)	6.48 (4.36)	7.883	**0.007** (0.125)	1.754	0.183 (0.06)
TA	6.32 (3.67)	5.00 (2.71)				
HA	6.85 (5.49)	6.26 (4.33)				
Total	6.48 (4.36)	5.45 (4.16)				
Depression						
TA + HA	1.95 (1.72)	2.84 (3.19)	0.034	0.854 (0.001)	0.152	0.86 (0.005)
TA	3.16 (3.88)	2.00 (2.45)				
HA	3.40 (3.52)	3.21 (3.75)				
Total	2.84 (3.19)	3.15 (3.36)				

Abbreviations: df, degrees of freedom; HA, hyaluronic acid; TA, triamcinolone acetonide; η^2, partial eta-square (effect size).
[a]Sphericity assumed.

size ($\eta^2 = 0.14$). The final sample size was 60 patients (20 per group).

A statistical analysis was performed using two-way ANOVA to measure the impact of factors related to the subject (observations at different visits) and external to the subject (type of treatment), as well as the interaction between the two. The differences in means for each treatment were analyzed, applying Bonferroni corrections in the comparisons. Everything was carried out with the SPSS 25.0 statistical software, considering a value of $p < 0.05$ as significant.

3 | Results

The randomized sample of the study was made up of 60 patients with symptomatic oral lichen planus, of which 58 patients completed the treatment (Figure 1). The ages of the patients included in the different treatment groups as well as the sex, and tobacco and alcohol consumption habits are homogeneous and are reflected in Table 1. We did not observe significant differences for any of the three study groups in all the variables.

Table 2 shows the results for the clinical variables of the Thongprasom, Desquamative Gingivitis study used by Arduino,

OHIP 14, and the HAD. The results showed that there was a significant change in scores over time, regardless of the group. Specifically, the scores of the Thongprasom, Arduino, OHIP 14, and anxiety scales (except AT group) decreased significantly at the end of treatment with respect to baseline values. In Table 3, the *p* values of the comparisons between pre- and posttreatment in each of the groups are shown. Regarding the pain VAS (Table 4), the results showed that there was a significant change in the scores throughout the study, regardless of the group. Specifically, pain decreased significantly at the end of treatment compared to baseline values in all groups (Figure 2). In general, all treatments seem to have a significant effect on the reduction in the VAS variable, which is maintained over time. The AH group shows continuous improvement between 2 weeks ($p = 0.002$) and 4 weeks ($p = 0.012$). No side effects were found in any of the study groups. The patients were satisfied with the treatment. The compliance rate was high, with only two dropouts (one in Group 1 and one in Group 2). These dropouts did not significantly affect the overall study results.

Regarding the salivary markers SOD, GSH, CUPRAC, FRAP, and TEAC, the results showed significant changes (Table 5). Specifically, the three groups were compared at three different times (start, T1 after cleaning and treatment assignment, and T2 after 4 weeks of treatment completion). Most of the salivary markers studied showed a significant decrease after cleaning, which could indicate that cleaning reduces oxidative stress or influences the antioxidant capacity of saliva.

After cleaning, we generally observe a slight increase or stabilization in the levels of the markers, which suggests a possible adaptation or positive response to the treatments over time (Table 6). In the analysis, we found for SOD (superoxide dismutase) that only the TA group showed a significant improvement in the SOD marker (TO–T1 value ($p < 0.001$) and HA group in T1–T2).

GSH (Glutathione). The TA group showed a significant improvement in T0–T1 ($p < 0.001$) and T1-T2 ($p = 0.035$). Furthermore, the HA group showed a significant change in T1–T2 ($p = 0.006$).

For cupric ion reduction capacity (CUPRAC), all groups showed a significant improvement T0-T1 TA-+HA ($p = 0.047$); TA $p = 0.006$; HA ($p < 0.001$). The TA group and the HA group also showed a significant change from T1 to T2 ($p = 0.009$ and $p = 0.013$). For FRAP (ferric reducing plasma antioxidant power) and TEAC (Trolox equivalent antioxidant capacity), all groups showed improvement from T0 to T1. We did not find statistically significant differences for any of the groups (T0-T2). There are no significant differences in the trend of these changes between the three treatment groups. This suggests that the effectiveness of the interventions in terms of modifying markers of oxidative stress is similar between the treatments evaluated.

TABLE 3 | *p* values for comparisons between baseline and after 4 weeks of treatment in each study group.

	TA + HA, *p* value	TA, *p* value	HA, *p* value
Thongprason	< 0.001	< 0.001	< 0.001
Arduino	0.002	0.042	0.037
OHIP-14	0.001	0.001	0.013
Anxiety	0.029	0.926	0.014
Depression	0.909	0.909	0.576

4 | Discusion

In the present study, topical application either as combination treatment (HA and TA) or as monotherapy, had a significant

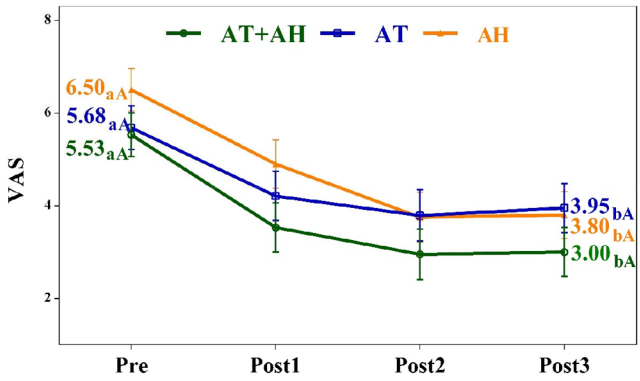

FIGURE 2 | Evolution of VAS scores according to the treatment. Pre-Baseline; Post1–14 days; Post2–4 weeks; Post3–3 months after treatment.

TABLE 4 | Means (SD) and statistical contrasts between groups regarding pain using the VAS scale.

	Time				Within-subject effects			
	T 0	T 14 days	T 4 weeks	T 3 months	Time		Treatment x time	
	Mean (ST)	Mean (ST)	Mean (ST)	Mean (ST)	*F*(3;165)	*p* value (η^2)	*F*(6;165)	*p* value (η^2)
VAS								
TA + HA	5.53 (1.84)	3.53 (1.87)	2.95 (2.20)	3.00 (1.94)	54.039	< 0.001 (0.496)	1.06	0.389 (0.037)
TA	5.68 (2.11)	4.21 (2.59)	3.79 (2.62)	3.95 (2.53)				
HA	6.50 (2.14)	4.90 (2.40)	3.75 (2.38)	3.80 (2.38)				
Total	5.91 (2.05)	4.22 (2.34)	3.50 (2.39)	3.59 (2.29)				

TABLE 5 | Means (SD) and statistical contrasts between groups for salivary markers.

	Time			Within-Subject Effects			
	To	T1	T2	Time		Treatment x time	
	Mean (ST)	Mean (ST)	Mean (ST)	$F(2;80)$	p value (η^2)	$F(4;80)$	p value (η^2)
SOD							
TA + HA	0.96 (0.84)	0.63 (0.67)	0.90 (0.63)	8.825	**< 0.001** (0.181)	2.411	0.056 (0.108)
TA	1.66 (1.43)	0.49 (0.21)	1.05 (0.77)				
HA	0.90 (0.66)	0.45 (0.26)	1.39 (1.77)				
Total	1.18 (1.08)	0.52 (0.43)	1.11 (1.16)				
GSH							
TA + HA	54.55 (51.45)	28.30 (21.05)	37.89 (21.62)	13.945	**< 0.001** (0.259)	1.461	0.222 (0.068)
TA	68.99 (46.54)	24.17 (9.42)	43.98 (32.51)				
HA	42.11 (30.02)	20.76 (7.62)	46.56 (39.38)				
Total	55.54 (44.10)	24.40 (13.94)	42.84 (31.47)				
CUPRAC							
TA + HA	0.38 (0.22)	0.20 (0.14)	0.40 (0.21)	17.573	**< 0.001** (0.305)	0.453	0.77 (0.022)
TA	0.41 (0.27)	0.19 (0.12)	0.44 (0.27)				
HA	0.50 (0.40)	0.18 (0.11)	0.43 (0.51)				
Total	0.43 (0.30)	0.19 (0.12)	0.42 (0.34)				
FRAP							
TA + HA	0.91 (0.52)	0.45 (0.30)	0.94 (0.41)	24.431	**< 0.001** (0.379)	0.729	0.575 (0.035)
TA	0.96 (0.59)	0.43 (0.29)	1.05 (0.60)				
HA	1.17 (0.94)	0.40 (0.26)	0.95 (0.90)				
Total	1.01 (0.70)	0.43 (0.28)	0.98 (0.66)				
TEAC							
TA + HA	0.43 (0.23)	0.23 (0.16)	0.45 (0.21)	25.952	**< 0.001** (0.394)	0.544	0.704 (0.026)
TA	0.48 (0.25)	0.21 (0.13)	0.48 (0.28)				
HA	0.53 (0.40)	0.19 (0.12)	0.44 (0.39)				
Total	0.48 (0.30)	0.21 (0.13)	0.46 (0.29)				

Note: To start: T1 Cleaning and Treatment Allocation T2 Treatment Completion in 4 Weeks.
Abbreviations: CUPRAC, cupric ion reducing antioxidant capacity; FRAP, ferric reducing antioxidant power; GSH, glutathione; HA Group, hyaluronic acid 0.1%; SOD, superoxide dismutase; TA Group, triamcinolone acetonide 0.2%; TA + HA, triamcinolone acetonide 0.2% + hyaluronic acid 0.1% group; TEAC, Trolox equivalent antioxidant capacity.

beneficial effect, including improving the quality-of-life indices, symptomatology assessed by the Thongprasom scale and visual analog scale (VAS), and the quality of life assessed by Oral Health Impact Profile-14 (OHIP14). The TA + HA combined group did not show a clear pattern, suggesting that the combination of treatments did not have significant additional effects. This lack of synergy could be due to drug interactions or a saturation of the mechanisms of action of the drugs, indicating that each agent acts sufficiently effectively on its own.

Anxiety and depression can have a negative impact on pain tolerance and modulation, which can influence pain perception and treatment efficacy in patients with oral lichen planus (OLP). Adamo et al. (2023) For this reason, we considered it relevant to include the HADS (Hospital Anxiety and Depression Scale) questionnaire in our study to evaluate these psychological aspects. We found statistically significant differences regarding anxiety and no significant differences regarding depression.

Hyaluronic acid is an important component in the extracellular matrix and is present in various body fluids. Its main properties are anti-inflammatory, bacteriostatic, fungicidal, proangiogenic, and tissue healing promotion (Waingade, Medikeri, and Gaikwad 2022). The findings of our study are partially consistent with those of previous research, which has shown that both HA and TA are effective in improving OLP symptoms.

TABLE 6 | Temporal changes in salivary oxidative stress markers during treatment with triamcinolone acetonide. Hyaluronic acid and their combination.

	To-T1	To-T2	T1-T2
SOD			
TA + HA	0.566	1	1
TA	< 0.001	0.187	0.222
HA	0.207	0.457	0.015
GSH			
TA + HA	0.05	0.531	0.669
TA	< 0.001	0.116	0.035
HA	0.146	1	0.006
CUPRAC			
TA + HA	0.047	1	0.053
TA	0.006	1	0.009
HA	< 0.001	1	0.013
FRAP			
TA + HA	0.021	1	0.009
TA	0.005	1	0.001
HA	< 0.001	0.673	0.003
TEAC			
TA + HA	0.021	1	0.007
TA	0.001	1	< 0.001
HA	< 0.001	0.803	0.002

Note: To start: T1 Cleaning and Treatment Allocation T2 Treatment Completion in 4 Weeks.
Abbreviations: CUPRAC, cupric ion reducing antioxidant capacity; FRAP, ferric reducing antioxidant power; GSH, glutathione; HA Group, hyaluronic acid 0.1%; SOD, superoxide dismutase; TA Group, triamcinolone acetonide 0.2%; TA + HA, triamcinolone acetonide 0.2% + hyaluronic acid 0.1% group; TEAC, Trolox equivalent antioxidant capacity.

For example, Shetty, Burde, and Guttal (2016) found that 0.2% HA significantly reduced symptoms and lesion size compared with placebo. However, our study provides a new perspective by showing that the combination of TA and HA does not increase this effectiveness, which is crucial for decision-making in clinical practice. Nolan et al. (2006) evaluated the effect of 0.2% HA gel in adults with ulcerative type OLP compared to a placebo. The results show that topical application of HA provided significant short-term pain relief, improving quality of life, although the analgesic effect was transient, disappearing approximately 4 h after application.

Hashem et al. (2019) evaluated the effect of 0.2% hyaluronic acid (HA) and triamcinolone in 40 patients. They found that HA reduced pain and clinical signs of oral lichen planus, but without significant differences in the degree of erythema between treatments. Santonocito et al. (2020) investigated the use of an anti-inflammatory mouthwash composed of calcium hydroxide (10%), hyaluronic acid (0.3%), umbelliferone, and oligomeric proanthocyanidins compared to clobetasol in 38 patients. It was found that clobetasol is the treatment of choice in the most severe forms of OLP. In parallel, Polizzi et al. (2023) compared this mouthwash versus topical tacrolimus in 38 patients. Both treatments were effective in reducing the signs and symptoms of OLP. However, the group treated with tacrolimus showed a more significant improvement compared with the mouthwash group. This could be due to the more potent immunomodulatory action of tacrolimus, which inhibits the activation of T lymphocytes, a crucial part in the pathogenesis of oral lichen planus. Therefore, the mouthwash, although effective, could be more suitable for less severe cases or as a maintenance treatment.

It is important to highlight the safety profile of HA, especially in terms of pain reduction and improvement in the appearance of oral lesions and quality of life. HA was well tolerated by patients, with an absence of significant adverse effects, reaffirming its safety profile for the long-term treatment of OLP. In our study, in line with others, we found that hyaluronic acid was well tolerated by patients, with no adverse effects. This suggests that it is a safe option for the long-term treatment of this chronic condition.

An emerging theory suggests that oxidative stress plays a key role in the pathogenesis of OLP (Wang et al. 2021; Gonzaga et al. 2023; Tvarijonaviciute et al. 2017). Studies have shown that patients with OLP have elevated levels of reactive oxygen species and reduced antioxidant activity in saliva (Tvarijonaviciute et al. 2017; Battino et al. 2008). These imbalances may contribute to both the inflammation and carcinogenic potential of OLP.

Our study also addresses the role of oxidative stress in OLP treatment. The results suggest that although the treatments improved OLP symptoms, there were no significant changes in oxidative stress biomarkers in saliva. Several studies have investigated SOD levels in patients with OLP, generally observing elevated levels compared with healthy controls (Azizi and Farshchi 2012). Thus, Darczuk et al. (2016) found that the mean salivary GSH concentrations in patients with OLP were significantly lower in the reticular and erosive groups compared with the control group, suggesting a deficiency in antioxidant defense.

We found that the levels of all markers tend to decrease after dental cleaning in all treatment groups. Regular maintenance and good hygiene can sustain and improve results by helping to keep inflammation and oxidative stress levels low in a more consistent way (Bianco et al. 2019; Garcia-Pola et al. 2019; Stone et al. 2015).

The influence of corticosteroids on oxidative stress is complex and can vary depending on the context (Wang et al. 2021; Gonzaga et al. 2023; Battino et al. 2008). Although corticosteroids can reduce oxidative stress in the short term due to their anti-inflammatory effects, their prolonged use can lead to an increase in oxidative stress due to side effects such as hyperglycemia and alteration of lipid metabolism. This underscores the importance of careful management of TA therapy to maximize its benefits while minimizing potential risks (Lodi et al. 2020).

The use of saliva to measure oxidative stress in OLP also has some limitations. Standardized processes for saliva collection time are still lacking, and if it is necessary to stimulate or centrifuge it after sampling, its temperature and storage time and analysis methods remain elusive (Waingade, Medikeri, and Gaikwad 2022). In general, it is important to have realistic expectations and understand that changes, such as SOD and TAC, take time. Maintaining a long-term focus and continuous evaluation is key to seeing significant improvements. We must consider the reasons for the absence of synergistic effects in this study, which could be due to saturation of the mechanisms of action (Chou 2006), since the components of the treatment, hyaluronic acid (HA), and triamcinolone (TA), could act on the same receptors or metabolic pathways. The proportion of HA and TA used in the study may not be ideal to obtain a synergistic effect.

The study's limitation includes the number of patients analyzed and the relatively short 4-week duration of treatment. Despite having used the Thongprasom scale, the lack of detailed data on the specific characteristics of the lesions (number of erosive lesions, involved sites, and presence of multiple lesions) is a limitation of our study. Furthermore, individual variability can influence the levels of these salivary biomarkers analyzed, so it is important to take into account other factors such as diet, lifestyle, and underlying health conditions. These results lead us to reflect on the need for personalized treatments based on the severity and individual response of patients to OLP. More research is needed to optimize its formulation, concentration, dosage, and route of application. Standardized assessment methods and identification of biomarkers could improve the reliability and clinical relevance of future studies.

5 | Conclusion

From the result of the study, it is concluded that the three application protocols were effective in treating symptomatic OLP, maintaining their effectiveness at 3 months of follow-up. However, no significant changes in oxidative stress markers in saliva were observed between the different groups.

Author Contributions

Rocio Rodriguez-Galvez: data curation, formal analysis, methodology, visualization, investigation. **Asta Tvarijonaviciute:** conceptualization, investigation, writing – original draft, methodology, formal analysis, supervision, project administration. **Camila Peres-Rubio:** investigation, methodology, data curation, formal analysis. **Pia Lopez-Jornet:** conceptualization, data curation, formal analysis, writing – original draft, methodology, supervision, investigation, project administration, validation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

References

Adamo, D., E. Calabria, F. Canfora, et al. 2023. "Anxiety and Depression in Keratotic Oral Lichen Planus: A Multicentric Study From the SIPMO." *Clinical Oral Investigations* 27, no. 6: 3057–3069. https://doi.org/10.1007/s00784-023-04909-3.

Alrashdan, M. S., N. Cirillo, and M. McCullough. 2016. "Oral Lichen Planus: A Literature Review and Update." *Archives of Dermatological Research* 308, no. 8: 539–551. https://doi.org/10.1007/s00403-016-1667-2.

Arduino, P. G., R. Broccoletti, V. Sciannameo, and C. Scully. 2017. "A Practical Clinical Recording System for Cases of Desquamative Gingivitis." *British Journal of Dermatology* 177, no. 1: 299–301.

Azizi, A., and F. Farshchi. 2012. "Comparison of Salivary and Plasma Antioxidant Levels in Lichen Planus Patients and Healthy Subjects." *Journal of Oral Pathology & Medicine* 41, no. 7: 524–526. https://doi.org/10.1111/j.1600-0714.2012.01138.x.

Battino, M., M. Greabu, A. Totan, et al. 2008. "Oxidative Stress Markers in Oral Lichen Planus." *BioFactors* 33, no. 4: 301–310. https://doi.org/10.1002/biof.5520330406.

Benzie, I. F., and J. J. Strain. 1996. "The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay." *Analytical Biochemistry* 239: 70–76.

Bianco, L., F. Romano, M. Maggiora, et al. 2019. "Effect of Sonic Versus Manual Supervised Toothbrushing on Both Clinical and Biochemical Profiles of Patients With Desquamative Gingivitis Associated With Oral Lichen Planus: A Randomized Controlled Trial." *International Journal of Dental Hygiene* 17, no. 2: 161–169. https://doi.org/10.1111/idh.12377.

Campos, C., R. Guzman, E. Lopez-Fernandez, and A. Casado. 2009. "Evaluation of the Copper (II) Reduction Assay Using Bathocuproinedisulfonic Acid Disodium Salt for the Total Antioxidant Capacity Assessment: The CUPRAC-BCS Assay." *Analytical Biochemistry* 392: 37–44.

Casale, M., A. Moffa, P. Vella, et al. 2016. "Hyaluronic Acid: Perspectives in Dentistry. A Systematic Review." *International Journal of Immunopathology and Pharmacology* 29, no. 4: 572–582. https://doi.org/10.1177/0394632016652906.

Chou, T. C. 2006. "Theoretical Basis, Experimental Design, and Computerized Simulation of Synergism and Antagonism in Drug Combination Studies." *Pharmacological Reviews* 58, no. 3: 621–681. https://doi.org/10.1124/pr.58.3.10.

Darczuk, D., W. Krzysciak, P. Vyhouskaya, et al. 2016. "Salivary Oxidative Status in Patients With Oral Lichen Planus." *Journal of Physiology and Pharmacology* 67, no. 6: 885–894.

Erel, O. 2004. "A Novel Automated Direct Measurement Method for Total Antioxidant Capacity Using a New Generation More Stable ABTS Radical Cation." *Clinical Biochemistry* 37: 277–285.

Garcia-Pola, M. J., L. Gonzalez-Alvarez, and J. M. Garcia-Martin. 2017. "Treatment of Oral Lichen Planus. Systematic Review and Therapeutic Guide." *Medicina Clínica (Barc)* 149, no. 8: 351–362.

Garcia-Pola, M. J., S. Rodriguez-López, A. Fernánz-Vigil, L. Bagán, and J. M. Garcia-Martín. 2019. "Oral Hygiene Instructions and Professional Control as Part of the Treatment of Desquamative Gingivitis, Systematic Review." *Medicina Oral, Patología Oral y Cirugía Bucal* 24, no. 2: e136–e144. https://doi.org/10.4317/medoral.22782.

Gonzaga, A. K. G., R. C. Vasconcelos, M. L. D. S. Lopes, et al. 2023. "Oxidative Stress Markers in the Saliva of Patients With Oral Lichen Planus." *Pathology Research and Practice* 248: 154569. https://doi.org/10.1016/j.prp.2023.154569.

Hashem, A. S., R. Issrani, T. E. E. Elsayed, and N. Prabhu. 2019. "Topical Hyaluronic Acid in the Management of Oral Lichen Planus: A Comparative Study." *Journal of Investigative and Clinical Dentistry* 10, no. 2: e12385. https://doi.org/10.1111/jicd.12385.

Lodi, G., M. Manfredi, V. Mercadante, R. Murphy, and M. Carrozzo. 2020. "Interventions for Treating Oral Lichen Planus: Corticosteroid Therapies." *Cochrane Database of Systematic Reviews* 2020, no. 2: CD001168. https://doi.org/10.1002/14651858.CD001168.pub3.

Louisy, A., E. Humbert, and M. Samimi. 2024. "Oral Lichen Planus: An Update on Diagnosis and Management." *American Journal of Clinical Dermatology* 25, no. 1: 35–53. https://doi.org/10.1007/s40257-023-00814-3.

Montero-Martín, J., M. Bravo-Pérez, A. Albaladejo-Martínez, L. A. Hernández-Martín, and E. M. Rosel-Gallardo. 2009. "Validation of the Oral Health Impact Profile (OHIP-14sp) for Adults in Spain." *Medicina Oral, Patología Oral y Cirugía Bucal* 14, no. 1: E44–E45.

Navazesh, M., and S. K. Kumar. 2008. "Measuring Salivary Flow: Challenges and Opportunities." *Journal of the American Dental Association* 139, no. Suppl: 35S–40S. https://doi.org/10.14219/jada.archive.2008.0353.

Nolan, A., C. Baillie, J. Badminton, M. Rudralingham, and R. A. Seymour. 2006. "The Efficacy of Topical Hyaluronic Acid in the Management of Recurrent Aphthous Ulceration." *Journal of Oral Pathology & Medicine* 35, no. 8: 461–465. https://doi.org/10.1111/j.1600-0714.2006.00433.x.

Polizzi, A., S. Santonocito, A. Lo Giudice, A. Alibrandi, R. De Pasquale, and G. Isola. 2023. "Analysis of the Response to Two Pharmacological Protocols in Patients With Oral Lichen Planus: A Randomized Clinical Trial." *Oral Diseases* 29, no. 2: 755–763. https://doi.org/10.1111/odi.13960.

Santonocito, S., A. Polizzi, R. De Pasquale, V. Ronsivalle, A. Lo Giudice, and G. Isola. 2020. "Analysis of the Efficacy of Two Treatment Protocols for Patients With Symptomatic Oral Lichen Planus: A Randomized Clinical Trial." *International Journal of Environmental Research and Public Health* 18: 56.

Shetty, R. R., K. N. Burde, and K. S. Guttal. 2016. "The Efficacy of Topical Hyaluronic Acid 0.2% in the Management of Symptomatic Oral Lichen Planus." *Journal of Clinical and Diagnostic Research* 10, no. 1: ZC46-ZC50. https://doi.org/10.7860/JCDR/2016/15934.7101.

Stone, S. J., P. A. Heasman, K. S. Staines, and G. I. McCracken. 2015. "The Impact of Structured Plaque Control for Patients With Gingival Manifestations of Oral Lichen Planus: A Randomized Controlled Study." *Journal of Clinical Periodontology* 42, no. 4: 356–362. https://doi.org/10.1111/jcpe.12385.

Thongprasom, K., L. Luangjarmekorn, T. Sererat, and W. Taweesap. 1992. "Relative Efficacy of Fluocinolone Acetonide Compared With Triamcinolone Acetonide in Treatment of Oral Lichen Planus." *Journal of Oral Pathology & Medicine* 21: 456–458.

Tvarijonaviciute, A., C. Aznar-Cayuela, C. P. Rubio, J. J. Ceron, and P. López-Jornet. 2017. "Evaluation of Salivary Oxidative Stress Biomarkers Nitric Oxide and C-Reactive Protein in Patients With Oral Lichen Planus and Burning Mouth Syndrome." *Journal of Oral Pathology & Medicine* 46, no. 5: 387–392. https://doi.org/10.1111/jop.12522.

Van der Meij, E. H., and I. van der Waal. 2003. "Lack of Clinicopathologic Correlation in the Diagnosis of Oral Lichen Planus Based on the Presently Available Diagnostic Criteria and Suggestions for Modifications." *Journal of Oral Pathology & Medicine* 32: 507–512.

Waingade, M., R. S. Medikeri, and S. Gaikwad. 2022. "Effectiveness of Hyaluronic Acid in the Management of Oral Lichen Planus: A Systematic Review and Meta-Analysis." *Journal of Dental Anesthesia and Pain Medicine* 22, no. 6: 405–417. https://doi.org/10.17245/jdapm.2022.22.6.405.

Wang, J., J. Yang, C. Wang, Z. Zhao, and Y. Fan. 2021. "Systematic Review and Meta-Analysis of Oxidative Stress and Antioxidant Markers in Oral Lichen Planus." *Oxidative Medicine and Cellular Longevity* 2021: 9914652. https://doi.org/10.1155/2021/9914652.

Warnakulasuriya, S., O. Kujan, J. M. Aguirre-Urizar, et al. 2021. "Oral Potentially Malignant Disorders: A Consensus Report From an International Seminar on Nomenclature and Classification Convened by the WHO Collaborating Center for Oral Cancer." *Oral Diseases* 27, no. 8: 1862–1884. https://doi.org/10.1111/odi.13704.

Wu, T., Y. Bai, Y. Jing, and F. Chen. 2024. "What Can We Learn From Treatments of Oral Lichen Planus?" *Frontiers in Cellular and Infection Microbiology* 14: 1279220. https://doi.org/10.3389/fcimb.2024.1279220.

Zigmond, A. S., and R. P. Snaith. 1983. "The Hospital Anxiety and Depression Scale." *Acta Psychiatrica Scandinavica* 67: 361–370.