



EVALUATION OF CHITOSAN AS A TREATMENT MODALITY FOR EROSIIVE ORAL LICHEN PLANUS VIA DETECTION OF SALIVARY TNF- α

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ABSTRACT

Objective: Oral lichen planus (OLP) is a common chronic inflammatory mucosal disease in which T-cell mediated immune responses are implicated in the pathogenesis. TNF has been found to be involved in the pathogenesis of many inflammatory or autoimmune diseases such as lichen planus. Various treatments have been employed to treat symptomatic OLP, but a complete cure is very difficult to achieve because of its recalcitrant nature. Topical corticosteroids therapy of OLP has shown conflicting results in many reports. Natural products have served as a major source of drugs for centuries, this study aimed to evaluate the effectiveness of topical intra-lesional chitosan in the treatment of oral erosive lichen planus. This was achieved via detection of salivary TNF- α level. **Subjects and methods:** The study was carried out using thirty-four patients of both sexes ranging in age between 30-50 years old with symptomatic erosive OLP and treated with intra-lesional 1% low molecular weight chitosan (50,000-190,000 Da) obtained from (Sigma-Aldrich, St. Louis, MO) and five healthy volunteers for estimation of the mean of normal salivary TNF- α . The appearance score, pain score, and salivary TNF- α of the target lesions were evaluated at weeks 0, 2, 4, and 16. **Results:** The study showed that there was a significant reduction of the mean of salivary TNF- α , VAS, score and criteria of clinical data which continued up to the end of the 4 months evaluation period as compared to the mean baseline value. **Conclusion:** Topical intra-lesional chitosan injection is effective for erosive OLP, which suggests that topical intra-lesional chitosan injection can be a promising therapeutic alternative for erosive OLP.

Keywords: Chitosan; Oral lichen planus; TNF- α .

INTRODUCTION

Oral lichen planus (OLP) is almost prevailing chronic autoimmune inflammatory disease of mucosal surface that manifest itself in an array of clinical symptoms of which are hyperkeratotic,

reticular, atrophic, papular, bullous forms, and erosive⁽¹⁾. The long-term erosive OLP is one of these syndromes opposes medicinal administration and mostly to be transformed into squamous cell carcinoma. As a result, OLP is viewed as a

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potentially malignant disorder that needs therapists' attention ⁽²⁾.

The TNF- α is a small cytokine having a MW of 17 kDa excreted by inflammatory cells (e.g., fibroblasts during infection or trauma and stimulated monocytes, macrophages, and several cells including B cells, T cells, mast cells). The cytokine which has multiple functions mediates immune response, inflammation, apoptosis as well as a magnificent function in natural homeostasis as well as development of other organs ⁽³⁾. TNF- α is implicated in the pathogenic of several inflammatory or autoimmune disorders, like systemic lupus erythematosus, psoriasis, rheumatoid arthritis, and OLP, according to studies ⁽⁴⁾.

The leading treatment for such a case involves the employment of high-potency topical corticosteroids ^(5,6). Topical corticosteroids are equal in effect or more effective than systemic corticosteroids and have fewer side effects ⁽⁷⁾. Topical corticosteroids when used for long time could also promote drug tolerance or insensitivity, adrenal insufficiency, pseudomembranous candidiasis, mucocutaneous atrophy, as well as Cushing's syndrome ⁽⁸⁾. Furthermore, a little percentage of people has allergy to corticosteroids ⁽⁹⁾. Others, on the other hand, are insensitive to corticosteroids or even immune to them due to mutations or polymorphisms in the corticosteroids receptor gene ⁽¹⁰⁾.

Medicinal plants act as an important source for pharmaceuticals. They provide almost half of known medications to humans ⁽¹¹⁾. Chitosan (poly-N-acetyl glucosaminoglycan), a chitin analogue, is one of these pharmaceuticals. The exoskeleton of arthropods (e.g. crustaceans, fungi cell wall, and insect's cuticle) is the second most abundant natural biopolymer. M-acetylation of chitin produces this chemical. It is biodegradable, biologically renewable, biocompatible, nontoxic, nonantigenic, and biofunctional. It hastens the healing of wound, enhances the functionality of macrophages, inflammatory cells, and fibroblasts ⁽¹²⁾.

The chitosan suppresses the genesis of PGE2 and cyclooxygenase-2 (COX-2) activation which get along with the suppression of TNF- α and the formation of IL-1 β but increasing of IL-10 generation ⁽¹³⁾. The release of a wide variety of anti-TNF agents is captured and stored by thermogelling chitosan/GP. This method of delivering disease-modifying therapy could result in the formation of a drug depository for treating local inflammation ⁽¹⁴⁾.

Treatment of OLP continues as a great defy for therapists. The aim of this research was to see if topical intralesional chitosan could help with OLP. This was achieved via detection of salivary TNF- α level.

SUBJECTS AND METHODS

A controlled clinical trial was implemented in the Dermatology Department, Al-Haud Al-Marsoud Hospital (Cairo, Egypt) with thirty-four patients of both sexes ranging in age between 30-50 years old. All were complaining of the typical criteria and a confirmed diagnosis of Erosive OLP were achieved. The treatment modality of the present study thoroughly explained and a written consent were obtained.

Selection criteria of participants

OLP clinically diagnosed (i.e., existence of a typical picture including painful and atrophic-erosive OL) with age range (30-50 years) and participants had the ability to finish the current experiment.

Dismission criteria of participants

Pregnant or lactating women and not taken any certain medications or had dental amalgam that can cause lichenoid reactions. Also, if was taken a previous OLP Therapy for the last 6 months preceding the study.

General criterion

All participants must be free from any systemic diseases according to Cornell Medical Index ⁽¹⁵⁾.

Grouping and intervention:

Thirty-four participants patients with Erosive-Atrophic O.L.P. as a study group. Five medically free volunteers for estimation the mean of salivary TNF- α in healthy condition. One% of low molecular weight (LMW) Chitosan was injected intralesional on weekly basis for a 3- 4 week-period (maximal of 0.1 ml /1 cm² of tissue/injection) ⁽¹⁶⁾.

Chitosan of MW 50-190 KDa was purchased from (Sigma-Aldrich, St. Louis, MO) and made as an aqueous solution with a concentration of 2% (in 1% acetic acid) at 48°C. A 45% aqueous solution beta glycerophosphate (β -GP) was included (drop by drop) to a 1:3 final volume ratio. The solution was kept fluid at sub physiologic temperature. One mL volumes each were included and employed to assess kinetics of sustained therapeutic release and gelation, which was, then, heated to physiologic temperature.

Clinical evaluation

The reactions of Erosive OLP to intralesional 1% LMW chitosan were assessed using erosive area and pain or the sensation of burning criteria. Throughout the trial, two blind independent researchers determined and reported erosive area, pain, and burning sensation values at baseline, 2, 4, and 16 weeks. The patient self-assessed the pressure or burning sensation using a 10-cm line visual equivalent scale (VAS), which is a well-documented form of pain assessment ⁽¹⁷⁾. Patients' current pain experience was rated on a scale of 0 (no pain) to 10 (extreme pain). The following graduated scale was used to measure the magnitude of pain and pain sensation:

0 Scale (VAS=0): no pain,

1st Scale 1 (0 < VAS \leq 3.5): mild pain,

2nd Scale (3.5 < VAS \leq 7): moderate pain,

3rd Scale (7 < VAS \leq 10): severe pain

Thongprasom et al, criteria ⁽¹⁸⁾ were used to rate the clinical results as follows:

5th Score for white striae having erosive region more than 1 cm.

4th Score for white striae having less than 1 cm of erosive area.

3rd Score for white striae having more than 1 cm of atrophic territory.

2nd Score for white striae having less than 1 cm of atrophic area.

1st Score for no erythematous field, mild white striae.

0th Score means that there is no lesion and the mucosa is fine.

Estimation of the salivary level of TNF- α

Two ml of whole un-activated saliva were collected at the same time before treatment and 2 weeks, 1 and 4 months after treatment using driving approach in disposable plastic tubes with lids that are sterile. The samples were centrifuged and the clear salivary fluids were separated and stored at -80°C till later analysis using ELISA technique ⁽¹⁹⁾.

Follow up assessment

Clinical improvement was assessed by the same scoring system after the therapy. Patients having completed vanishing of the erosion at all times were checked up to see the recurrence. Meantime, patients who did not fully respond were evaluated on weekly basis for three weeks more. When there was no sign of erosion and the VAS was nil, a complete stoppage of the treatment imposed.



FIG (1): a&b) The buccal mucosa showed erythematous and ulcerative which was more intensive in right side, c&d) Clinical response after 2 weeks of treatment with 1% of chitosan, e) Intralesional chitosan injection in erosive area, f&g) Clinical response after 1 month of treatment with 1% of chitosan, h&i) Clinical response after 4 months of treatment with 1% of chitosan.

RESULTS

The current investigation included thirty-four patients of erosive OLP in study group. The demographic distribution of the patients is present in Table (1).

Results indicated a significant decrement in the average of salivary TNF- α , VAS, score and criteria of clinical signs (erosion, appearance score) of the whole evaluation period (4 months) in comparison with the mean baseline values.

The average difference in salivary TNF- α , VAS, ranking, and clinical parameters at baseline, on the other hand, was statistically insignificant.

TABLE (1): Distribution of the studied cases according to demographic data

	No.	%
Sex		
Male	15	44.2
Female	19	55.8
Age (years)		
Min. – Max.	33.0 – 49.0	
Mean \pm SD.	40.96 \pm 4.49	
Median	40.50	

Table (2) shows a comparison between different time periods in test group with regard to VAS. Test group displayed a statistically a significant decrement in mean VAS measurements at 2 weeks, 1, 4 months.

Table (3) shows a comparison for various time spans in test group with regard to Thongprasom score. Test group exhibited a statistically a significant decrease in mean Thongprasom score measurements at 2 weeks, 1, 4 months.

Table (4) contrasts the various time spans in the control community in terms of clinical progress.

There was a statistically significant clinical improvement (58.8% of cases) from Baseline to 2 weeks. There was a statistically significant clinical improvement (100.0% of cases) from 2 week, till 1 and 4 months.

In terms of TNF- α , Table (5) summarizes the distinction between the various time periods in the test sample. Mean TNF- α at baseline was 34.08 ± 6.86 pg/ml, at 2 weeks was 21.82 ± 4.82 pg/ml, at 1 month was 13.14 ± 3.04 pg/ml, and at 4 months was 7.34 ± 2.11 pg/ml. A statistical significance existed in the decrement in TNF- α after 2 week, 1 and 4 months ($p < 0.001^*$).

Table (2): Comparison between the different time periods in test group with regard to VAS.

	VAS				Fr	p
	Baseline	2 weeks	1 months	4 months		
Test group	8.83 \pm 1.01	5.13 \pm 1.42	1.58 \pm 0.97	0.75 \pm 0.74	70.615*	<0.001*
P_{Baseline}		0.007*	<0.001*	<0.001*		

Fr: Friedman test,

Post Hoc Test (Dunn's) for Sig. between periods

p_{Baseline} represents (P) value for the comparison between **baseline** and each other period in test group.

TABLE (3): Comparison between the different time periods in test group with regard to Thongprasom score.

	Thongprasom Score				Fr	p
	Baseline	2 weeks	1 months	4 months		
Test group	4.83 \pm 0.38	3.17 \pm 0.82	1.21 \pm 0.59	0.67 \pm 0.48	70.127*	<0.001*
P_{Baseline}		0.007*	<0.001*	<0.001*		

Fr: Friedman test,

Post Hoc Test (Dunn's) for Sig. between periods

p_{Baseline} represents (P) value for the comparison between **baseline** and each other period in test group.

TABLE (4): A comparison of various time spans in test group with regard to clinical improvement.

	Clinical improvement				Q	p
	Baseline	2 weeks	1 months	4 months		
Test group						
No	34 (100.0%)	14 (41.2%)	0 (0.0%)	0 (0.0%)	42.0*	<0.001*
Yes	0 (0.0%)	20 (58.8%)	34 (100.0%)	34 (100.0%)		
P_{Baseline}		<0.001*	1.000	1.000		

Q: Cochran's test,**Post Hoc Test (Dunn's) for Sig. between periods**

$P_{Baseline}$: p value for comparing between **baseline** and each other period in test group.

TABLE (5): A comparison of various time spans in test group with regard to TNF- α .

	TNF- α (pg/ml)				F	p
	At baseline	2 weeks	1 months	4 months		
Test group						
	34.08 \pm 6.86	21.82 \pm 4.82	13.14 \pm 3.04	7.34 \pm 2.11	266.127*	<0.001*
P_{Baseline}		<0.001*	<0.001*	<0.001*		

F: F test (ANOVA) with repeated measures, Sig. bet. periods were done using**Post Hoc Test (adjusted Bonferroni)**

$P_{Baseline}$: p value for comparing between **baseline** and each other period in the study group.

DISCUSSION

The administration of erosive lichen planes is still a challenge. Current remedies largely supply symptom relief rather than absolution. In the treatment of mild to moderate symptomatic lesions, topical corticosteroids are the cornerstone (20). Topical steroid administration has less side effects than systemic steroid administration. The abuse of corticosteroids include: discomfort while applying, compliance problem, thinning of the oral mucosa, and candidiasis. When used in large doses and over long periods of time, topical formulations of the more powerful corticosteroids can cause adrenal suppression (1).

Corticosteroids are not indicated to patients practicing breast feeding, but administered with

care in patients having glaucoma, HIV infection, herpetic infections, tuberculosis, pregnancy, candidiasis, diabetes mellitus, and hypertension (21). Due to such clinical complications and because of the immuno-pathogenesis of OLP, this investigation explored the topical immuno-modulatory drug for therapy of this disorder as alternate if topical corticosteroids are fruitless or contraindicated. The current investigation was implemented to evaluate the effectiveness of intra-lesional chitosan for erosive lichen planes to be used as an alternative therapeutic agent and to support the results, salivary TNF- α was measured throughout the treatment.

TNF- α - a pro-inflammatory cytokine - contribute the pathogenesis and inflammation process of OLP (3). TNF- α was found to be low in

the analyzed group compared as compared to the baseline values and with its average mean value obtained from the five healthy volunteers in the current study. The anti-inflammatory properties of chitosan are responsible for the decrease. It suppresses prostaglandin E2 (PGE2) production through inhibiting cyclooxygenase (COX2) stimulation and action, as well as TNF- α and the IL-1 β production. Furthermore, it boosts the anti-inflammatory cytokine IL-10^(13,22).

Shamji et al.⁽¹⁴⁾ demonstrated chitosan's ability to release a wide variety of anti-TNF- α agents as well as the suppressive effect of chitosan on TNF- α agents. They also showed the suppressive effect of chitosan against TNF- α . As compared to baseline levels, the tested group exhibited a substantial decrease in medical manifestations (erosion, appearance score) that was sustained until the research period is over.

The anti-inflammatory effect of chitosan may explain why clinical symptoms are reduced when it's present^(13,22). The development of granulation tissue and angiogenesis in the ulcerated area of the stomach in rats was accelerated by repeated oral administration of LMW chitosan⁽²³⁾.

OLP is currently being treated with the aim of relieving pain and removing the lesions. At all assessment times in the current study, the analyzed group had a decrease in clinical manifestations (pain, burning sensation) (VAS score) when compared to the baseline's high score. Chitosan offered excellent pain relief when applied topically to open wounds (burns, skin abrasions, skin ulcers, and skin graft areas) because proton ions released from the inflammatory site is absorbed, whereas the main analgesic effect of chitin is the absorption of bradykinin, the main substance linked to pain⁽²²⁾.

In addition, when compared to the baseline situation of the lesions, the tested group showed an improvement in clinical signs about lesion

size (Thongprasom score). Chitosan inhibited extracellular matrix degradation, increased type I collagen expression, and increased alkaline phosphatase activity⁽²⁴⁾.

Finally, for erosive OLP, topical intralesional chitosan injection is successful, suggesting that it may be a favorable remedial option for erosive OLP, in particular those who are intolerant or invulnerable to corticosteroids. Furthermore, the administration of 1% LMW chitosan for the treatment of OLP should be supported, studied in larger RCTs either by itself or in combination with other therapeutic agents.

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