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# Ozone Therapy Versus Topical Hyaluronic Acid- Triamcinolone Acetonide- Diclofenac Sodium In Treatment of Recurrent Aphthous Stomatitis

Zekayi Kutlubay,[1] MD, Muazzez Çiğdem Oba,[1*] MD, Burhan Engin,[1] MD, Pınar Ambarcığlu,[2] MD, Server Serdaroğlu,[1] MD, Yalçın Tüzün,[1]MD

*Address:* [1]Istanbul University, Cerrahpaşa Medical Faculty, Department of Dermatology and Venereology, Istanbul. [2]Faculty of Veterinary Medicine, Ankara University, Ankara.

*E-mail:* muazzez.oba@istanbul.edu.tr

**Corresponding Author*: Dr.Muazzez Çiğdem Oba, Istanbul University, Cerrahpaşa Medical Faculty, Department of Dermatology and Venereology, Istanbul.

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**Keywords:** Hyaluronic acid-triamcinolone acetonide-diclofenac sodium, ozone, recurrent aphthous stomatitis

**Abstract**

**Background:** Recurrent aphthous stomatitis (RAS) is a common mucosal disorder presenting as painful ulcerations in oral cavity. As the etiology is not fully understood, there is no cure of the disease and treatment of RAS is largely aimed at symptom control. The purpose of this study was to compare the therapeutic efficacy of ozone therapy with conventional topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium therapy in the treatment of patients with recurrent aphthous stomatitis.

**Material and Methods:** The clinical trial was carried out on 40 patients with RAS divided into two groups. Group 1 (n=20) was prescribed topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium two times daily for one week, while Group 2 (n=20) was treated with ozone by local insufflation of the gas at two consecutive days. Treatment efficacy was assessed by comparing pain scores and greatest diameter of the lesion in both groups at baseline and after one week.

**Results:** In Group 1 pre-treatment mean pain score was 3,85 ± 1,23; the mean pain score at 1st week was 0,60 ± 1,14. In Group 2 pre-treatment mean pain score was 3,05 ± 1,73; the mean pain score at 1st week was 0,16 ± 0,37. In Group 1 pre-treatment lesion size of 3,20 ± 1,32 regressed to 0,55 ± 0,89 at 1st week. In Group 2 pre-treatment lesion size of 3,89 ± 2,06 regressed to 0,42 ± 1,04 at 1st week. Ozone therapy showed better results than topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium in reduction of pain scores. However, ozone therapy and topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium application both proved to be similarly effective on reducing the size of the lesions.

**Conclusion:** Ozone therapy could be considered as an alternative treatment modality in the management of patients with recurrent aphthous stomatitis.

## Introduction

Recurrent aphthous stomatitis (RAS) is a common mucosal disorder characterized by rounded or ovoid painful ulcerations in oral cavity [1]. Affecting %20 of the population, RAS is the most common ulcerative disease of oral mucosa. Genetic, immunological, hormonal and microbial factors have all been suggested as predisposing factors [1]. As the

etiology of RAS is unknown, there is no cure of the disease and treatment of RAS is largely aimed at reducing pain. Conventional treatment agents are topically applied antiseptics, analgesics, corticosteroids and antibiotics. In severe cases, systemic immunomodulators such as azathioprine, colchicine, cyclosporine may be used. [**1,2**]

Ozone therapy is the application of a certain amount of ozone/oxygen mixture into body cavities or circulation. It has immunostimulant properties and facilitates oxygen delivery to the tissues [**3,4**].

In this study, our aim was to compare the therapeutic efficacy of ozone therapy with conventional topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium therapy in the treatment of patients with recurrent aphthous stomatitis.

## Methods

The clinical trial was performed in accordance with the principles of the Declaration of Helsinki. All of the subjects were given necessary information regarding the characteristics of the study and gave their written consent before being enrolled into the trial.

40 patients of either sex (20 men and 20 women) aged between 15 and 55 years old were recruited into the study. The study was undertaken during the period from April 2011 until December 2016. The participants were retrospectively selected from the patients who had applied to Istanbul University, Cerrahpasa Medical Faculty, Dermatology and Venereology department outpatient clinic with a complaint of oral ulcers. Diagnosis of recurrent aphthous stomatitis was made on the basis of history and clinical symptoms. Patients with less than 4 lesions per year, patients with a diagnosis of any systemic disease or herpes labialis, patients under treatment with topical or systemic steroids or immunomodulatory agents, patients who were pregnant or breast feeding were excluded from the study. At the first visit, demographic information, history of the episodes and duration of current lesion were noted.

40 patients were randomly divided into two equal groups. In patients with multiple oral aphthae, the most painful ulcer reported by the patient was selected as the index ulcer for the study. Subjects in Group 1 applied topical hyaluronic acid- %0.1 triamcinolone acetonide-diclofenac sodium (over-the-counter product) two times daily for one week; while subjects in Group 2 received ozone therapy by local insufflation of the gas at two consecutive days. By means of a glass probe, the ulcer was subjected to ozone gas for 40 seconds. Treatment efficacy was assessed by comparing pain scores and ulcer size in both groups at baseline and after one week. Pain intensity was recorded by using a 6-point numeric rating scale where “0” was no pain and “5” the worst pain. Ulcer size was assessed by measuring the greatest diameter of the lesion. Ulcers of the patients were photographed before the start of treatment and one-week post treatment.

Statistical analysis was carried out using the SPSS computer software (Statistical Package for the Social Sciences; version 14.01). Independent samples t test was carried out to compare demographic variables among treatment groups. One - way analysis of covariance was conducted for this study to compare post-treatment scores among treatment groups after adjustment for pre-treatment scores. Since the pre and post-treatment pain score and pre and post-treatment lesion size variables contained many zero values, these variables were transformed by log(x+1) transformation before conducting one-way analysis of covariance. P value of <0,05 was considered to indicate statistically significance for all comparisons.

## Statistical evaluation

In the statistical evaluation of the data, the SAS 9.12 statistical software program was used. For examination of the variables in the study, firstly descriptive statistical calculations were made. Then, to establish differences between the characteristics of the groups such as age, gender, duration of the disease, lesion type, localization and diameter, the proportions test was used. A value of $p<0.05$ was accepted as statistically significant.

## Results

There were 10 males and 10 females in both Group 1 and Group 2. Mean age of subjects in Group 1 was 31,70 ± 8,17 and in Group 2 was 34,10± 10,48 years. Duration of the disease was 7,00± 5,04 years in Group 1 and 6,40±4,66 years in Group 2. Duration of the current lesion was 4,55±2,65 days

**Table 1.** Characteristics of the patients in Group 1 and Group 2

| | | Treatment | | p |
|---|---|---|---|---|
| | | **Group 1** | **Group 2** | |
| | | **n(%)** | **n (%)** | |
| Sex | Female | 10 (50) | 10 (50) | 1 |
| | Male | 10 (50) | 10 (50) | |
| | | **Mean ± SD** | **Mean ± SD** | |
| Age | | 31,70 ± 8,17 | 34,10 ± 10,48 | 0,425 |
| Disease duration | | 7,00 ± 5,04 | 6,40 ± 4,66 | 0,698 |
| Lesion duration | | 4,55 ± 2,65 | 5,75 ± 1,94 | 0,111 |

in Group 1 and 5,75±1,94 days in Group 2. Table 1 summarizes the characteristics of the patients enrolled in the study. There were no significant differences between the two groups regarding patients' age, gender, disease duration and lesion duration (**Table 1**).

In Group 1 pre-treatment mean pain score was 3,85 ± 1,23; while the mean pain score at 1st week was 0,60 ± 1,14. In Group 2 pre-treatment mean pain score was 3,05 ± 1,73; while the mean pain score at 1st week was 0,16 ± 0,37. In Group 1 pre-treatment lesion size of 3,20 ± 1,32 regressed to 0,55 ± 0,89 at 1st week. In Group 2 pre-treatment lesion size of 3,89 ± 2,06 regressed to 0,42 ± 1,04 at 1st week. Table 2 shows pre-treatment and post-treatment pain scores and lesion size in both Group 1 and Group 2. None of the patients had any side effects. In both groups, mean pain score and lesion size were reduced (**Table 2**).

The covariate, pre-treatment pain score, was significantly related to the post-treatment pain score. ($F(1,36)=151,986$ $p<0,001$) There was significant effect of treatment group on post-treatment pain score after adjusting for the pre-treatment pain score. ($F(1,36)=4,444$ $p<0,05$). The covariate, pre-treatment score of lesion size, was significantly related to the post-treatment score of lesion size ($F(1,37)=6,341$ $p<0,05$). But there was not significant effect of treatment group on post-treatment lesion size after adjusting for the pre-treatment lesion size. ($F(1,37)=1,077$ $p>0,05$)

Ozone therapy showed better results than topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium in reduction of pain scores (p: 0,042). However, ozone therapy and topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium application both proved to be similarly effective on reducing the size of the lesions (**Table 3**).

## Conclusions

Ozone (O3) is an allotropic form of oxygen with diverse biological effects. It is a powerful oxidant, has strong antimicrobial activity against bacteria, viruses, funghi and protozoa, and has antihypoxic, immunostimul ating and analgesic properites. By these unique properties ozone therapy has been st udied in various fields of medicine and recommended for the treatment of 260 different pathologies [**5, 6**].

In dentistry ozone is used as an antiseptic in order to reduce caries-related pathogens, disinfection of root canals but also to improve epithelial wound healing. It has been shown to be safe with less toxic effects on human oral epithelial cells and gingival fibroblast cells than chlorhexidine, sodium hypochlorite or hydrogen peroxide, agents commonly used in clinical practice [**7**].

Ozone therapy has been used for the treatment of skin ulcers, pressure sores, fistulae etc [**8**]. In a study, ozone autohemotherapy performed for several months in two patients with chronic leg ulcers was reported to achieve satisfactory healing [**9**]. Martinez- Sanc-

**Table 2.** Changes observed in pain scores and lesion size in Group 1 and Group 2

| | Treatment | N | Mean | Standard Deviation | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|---|
| Pain score (Pre-Treatment) | Group 1 | 20 | 3,85 | 1,23 | 4 | 1 | 5 |
| | Group 2 | 20 | 3,05 | 1,73 | 4 | 0 | 5 |
| Pain score (Post-Treatment) | Group 1 | 20 | 0,6 | 1,14 | 0 | 0 | 4 |
| | Group 2 | 20 | 0,15 | 0,37 | 0 | 0 | 1 |
| Lesion size (Pre-Treatment) | Group 1 | 20 | 3,2 | 1,32 | 3,5 | 1 | 5 |
| | Group 2 | 20 | 3,89 | 2,06 | 4 | 0 | 8 |
| Lesion size (Post-Treatment) | Group 1 | 20 | 0,55 | 0,89 | 0 | 0 | 4 |
| | Group 2 | 20 | 0,42 | 1,04 | 0 | 0 | 2 |

**Table 3.** Adjusted pain scores and lesion size in Group 1 and Group 2

| Effect | | Mean± SD | Estimated Marginal Mean ± SE | F | df | p |
|---|---|---|---|---|---|---|
| Pain score (log) (Pre-Treatment) | | | | 151,986 | 1 | **<0,001** |
| Group | Group 1 | 0,134±0,228 | 0,116±0,018 | 4,444 | 1 | **0,041** |
| | Group 2 | 0,045±0,110 | 0,063±0,018 | | | |
| Error | | | | | 36 | |
| Lesion size (log) (Pre-Treatment) | | | | 6,341 | 1 | **0,016** |
| Group | Group 1 | 0,134±0,214 | 0,144±0,044 | 1,077 | 1 | 0,306 |
| | Group 2 | 0,088±0,205 | 0,079±0,044 | | | |
| Error | | | | | 37 | |

hez et al. reported prevention of oxidative stress underlying the diabetic complications by use of ozone therapy. 52 patients with di abetic foot treated with ozone showed better healing of ulcers and less amputation needs compared to 49 patients under topical and systemic antibiotic treatment [**10**]. In another article, management of diabetic foot ulcers by means of combination of ozone treatment with conventional treatment was associated with better results than conventional treatment alone in obtaining total wound closure [**11**].

Use of topical ozone for the treatment of RAS has been compared with placebo in previous studies. Dharmavaram AT et al. have reported ozonated oil to be more effective than sesame oil and placebo in relieving ulcer pain and reducing ulcer size and erythema [**12**]. In a murine model, topical ozonated sesame oil application was shown to accelerate cutaneous wound closure [**13**]. In our study, we applied ozone gas on RAS lesions by using Biozonix machine. The medical ozone generators produce ozone from oxygen. Therefore, a gas mixture of oxygen and ozone is collected [**14**]. In the observational study conducted by Al-Olmiri et al. ozone was applied for 60 seconds on RAS lesions by healOzone X4 machine. Ulcers healing duration and ulcers size were significantly reduced compared to placebo [**15**].

Limitations of the study were that the sample size was small. We think that since many RAS patients receive first line conventional symptomatic treatment by family physicians, our tertiary institution did not have frequent applications with this complaint. Also, pain score recordings depended on subjective patient reports.

Our study demonstrated that ozone therapy was associated with better results than the conventional topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium treatment in reduction of pain scores. However, ozone therapy and topical hyaluronic acid-triamcinolone acetonide-diclofenac sodium application both proved to be similarly effective on reducing the size of the lesions. As patients are distressed mostly by painful nature of the lesions, ozone therapy with its better analgesic effects could be considered as an alternative treatment option in management of RAS. Further large-scale studies are required to justify the use of ozone therapy for the treatment of RAS.

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