



## EVALUATION OF THE EFFECTIVENESS OF OZONIZED OLIVE OIL IN THE TREATMENT OF ORAL WHITE & RED AND ULCERATIVE LESIONS

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### ABSTRACT

The oral cavity is an open ecosystem that shows a dynamic balance between the entrance of microorganisms (consist of bacteria, virus, fungal etc), colonization modalities, nutritional balance and host defences against their removal. In our day to day clinical practice we are encountered with different oral mucosal lesion such as aphthous ulcer, angular cheilitis, oral lichen planus, candidiasis. There are variety of treatment options available in the literature for all of these oral mucosal lesions. Topical ozone therapy is highly valued and minimally invasive technique as ozone has antimicrobial, anti-hypoxic, analgesic, and analgesic and immunostimulant effect. Aim & Objectives: Evaluation of the effectiveness of ozonized olive oil in the treatment of oral white & red and ulcerative lesions. Materials and Methods: A longitudinal study was carried out in 60 patients (19 to 65 years, both male & female). All the patient will clinically examined & diagnosed for the respective lesions (aphthous ulcer, angular cheilitis, oral lichen planus & candidiasis) and intergroup comparison of data was done by using ANOVA test (mean days of complete healing of respective lesions) Result: All the lesions regress in patients with aphthous ulcer, angular cheilitis, oral candidiasis and improvement in sign and symptoms in oral lichen planus patients. No side effects were observed in any patient. Conclusion: Topical ozone therapy is quite effective in treating oral mucosal lesion. Hence, it can be considered as a minimally invasive therapy for the oral infective and immunological conditions.

**Keywords:** Oral mucosal lesion, ozone therapy, topical O<sub>3</sub>, OLP, Aphthous Ulcer, Angular Cheilitis, Candidiasis

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## INTRODUCTION

The problem for treatment of erosive & ulcerative lesions of the oral cavity is relevant for dentists of all directions due to the chronization of the process. Various therapeutic approaches have been proposed, but definite cure for these lesions entity remains still untouched. The large number of therapeutic agents including corticosteroids, retinoid, calcineurin inhibitors, laser & photodynamic therapy etc.

Numerous different topical & systemic treatments have been suggested till now for relieving pain or eliminating the sign & symptoms of the lesions with minimum or no side effects. These serves as one of the cornerstone in the maintenance of the biodynamic of the body with various actions like antioxidant, anti-inflammatory, anti-analgesic etc. Ozone therapy has gained a prominent consideration in the medical and dental fields due to its physiochemical properties. It possesses strong antimicrobial activity, anti-hypoxic and immunostimulant, anti-inflammatory. Ozone therapy is an alternative non-medication therapy that has also been introduced as a treatment option. Ozone application in form of oil used due to its cost-effective, easy manipulation nature and better shelf-life of the medication. [1]

Ozone is present in the environment as a protective gaseous layer. It prevents living beings from the harmful effects of the high energy ultraviolet radiations. It was discovered in 1839 by Christian Friedrich Schonbein when he noticed the emergence of a pungent gas with an electric smell. The word ozone is derived from Greek word "ozein" that means odor, named after its characteristic odor usually detected by human nose at 0.02 ppm or 0.05 ppm. Since then it has been the field of research & clinical application in medicine & dentistry. The first medical application of ozone was traced back to 1870 by Dr. C. Lender, who used it for purifying blood in test tubes. [2]

Ozone also called trioxxygen is an allotropic form of oxygen & an unstable gas which occurs naturally by the action of ultraviolet rays on oxygen in the outer atmosphere. It is known to be a potent oxidizer & has the ability to oxidize any known biological entity. The principal action of ozone is its antimicrobial effect on bacteria, virus & fungi, besides its immunomodulatory, anti-hypoxic, biosynthetic & anti-inflammatory properties. [3]

Ozone has been accepted as an alternative & complimentary therapy. Medical-grade ozone is essentially a composition of 0.1 – 5% ozone & 95-99.6% pure oxygen & in dentistry it has been used either as a gas, water or oil to treat a plethora of conditions.

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Use of ozone in dental therapeutics is truly a paradigm shift in the management of oral & dental infections. [3]

Ozone kills all kinds of bacteria, viruses, fungi & protozoa. The antiseptic effect of pure ozone is three hundred times stronger than that of chlorine. Ozone has no destructive & irritating effect on tissues. Therapeutic doses of ozone have an anti-hypoxic, immunocorrective effect, potentiate the action of antibiotics, improve the rheological properties of blood & enhance microcirculation. [4]

Ozone therapy increases the delivery of oxygen to tissues, inhibits lipid peroxidation & activates the antioxidant system in the lesion. One of the important properties of ozone is its antibacterial effect and the absence of a selective effect for antibiotic-resistant strains as well as the absence of other consequences of traditional drug antibiotic therapy. [4]

## MATERIALS AND METHODS

A total of 60 patients of age & sex (19 to 65 years; both male & female) enrolled from the year 2020 to 2022 were recorded in a proforma. All the patients were clinically examined & diagnosed for the respective lesions and intergroup comparison of data was done by using ANOVA test (mean days of complete healing of respective lesions).

## INCLUSION CRITERIA

- a. Patient having Oral lichen planus
- b. Patient having oral candidiasis
- c. Patient having aphthous ulcer
- d. Patient having Angular cheilitis
- e. Age ranging from 19 to 65 years.

## EXCLUSION CRITERIA

- a. Pregnancy/lactating mother
- b. History of surgical and non-surgical therapy 6 months prior to the study
- c. Patient on antibiotic therapy
- d. Patient undergoing chemotherapeutic treatment
- e. Patient Allergic to ozonized olive oil

## ARMAMENTARIUM USED

- Mouth mirror
- Tweezers
- Probe
- Kidney tray
- Sterile gauge pieces
- Sterile cotton plug
- Normal water
- Latex gloves
- Venier calliper
- Ozonized olive oil

**METHOD**

1. Patients were clinically examined for the above mentioned lesions.
2. A written informed consent from all the patient were taken.
3. Patient were asked to rinse the mouth with water.
4. The part of the oral mucosa with the lesion were isolated with sterile gauge & ozonized olive oil were applied topically over the lesions with the help of sterile cotton plug or gloved finger.
5. The viscous oil was massaged over the lesion for 1 min.
6. The patients were advised not to have anything to eat or drink for half an hour.
7. The application was done twice daily till the time lesions subsides, to a maximum period of 6 months duration.

8. All the patient was recalled weekly for follow up visit after the regression of lesions for a maximum period of 6 months.
9. At every recall visit, patient's signs & symptoms such as burning sensation were noted in the records along with the size of the lesions.

**RESULTS**

The study was aimed to evaluate the effectiveness of ozonized olive in the treatment of

- White & red lesions (Oral lichen planus & Oral candidiasis)
- Ulcerative lesions of oral cavity (Apthous ulcer & Angular cheilitis)

The study comprised total of 60 subject (each group consist of 15 subject) who were selected after performing inclusion and exclusion criteria.

**TABLE 1: Comparison of elimination of burning sensation after applying ozonized olive oil among all test groups**

S.N.	TEST GROUP	NUMBER	Burning elimination in Mean Days (Mean ± S.D.)	F value	p value
1.	Group A (Oral Lichen Planus)	15	7.33± 0.81	369.4	0.000 (P<0.001) Very Highly Significant
2.	Group B (Apthous Ulcer)	15	1.33± 0.42		
3.	Group C (Angular Cheilitis)	15	1.46±0.51		
4.	Group D (Oral Candidiasis)	15	1.73± 0.45		

**TABLE 2: Multiple Pairwise Comparison of Mean Days in which burning sensation is eliminated on Ozonized olive oil application after applying Post Hoc (Tukey) test**

Groups Comparison		Mean Difference	P value
<b>Group A</b>	Group B	6.0000*	0.000(<0.001) (HS)
	Group C	5.86667*	0.000 (<0.001) (HS)
	Group D	5.60000*	0.0000(<0.001) (HS)
<b>Group B</b>	Group A	-6.00000*	0.000(<0.001) (HS)
	Group C	-.13333	0.925(>0.05) (NS)
	Group D	-.40000	0.255 (>0.05) (NS)
<b>Group C</b>	Group A	-5.86667*	0.000(<0.001) (HS)
	Group B	.13333	0.925(>0.05) (NS)
	Group D	-.26667	0.603 (>0.05) (NS)
<b>Group D</b>	Group A	-5.60000*	0.000(<0.001) (HS)
	Group B	.40000	0.255(>0.05) (NS)
	Group C	.26667	0.603 (>0.05) (NS)

**Table 3: Comparison of Mean days of complete healing after applying ozonized olive oil among all test groups**

Group	Healing after Mean Days (Mean ±S.D.)	F value	P value
Group B (Apthous Ulcer)	2.26±0.45	23.43	0.000 (P<0.001) Very Highly Significant
Group C (Angular Cheilitis)	2.33±0.44		
Group D (Oral Candidiasis)	<b>3.34±0.48</b>		

**Table 4: Multiple Pairwise Comparison of Mean Days of Healing among all the groups after applying Post Hoc (Tukey) Test**

Group B	On Day 0	After 1 Days	After 2 Days
Mean Size (in mm <sup>2</sup> )	5.14	2.47	1.98
S.D.	1.29	1.22	1.21
Group B	On Day 0	After 1 Days	After 2 Days
Mean Size(in mm <sup>2</sup> )	5.14	2.47	1.98
S.D.	1.29	1.22	1.21

**Table 5: Comparison of Mean Size in mm<sup>2</sup> in Group A (Oral Lichen Planus) Lesions after 0 day, 7 day, 14 days, 1 month, 2months, 3 months, 6 months**

Group A	On Day 0	After 7 Days	After 14 Days	After 1 Month	After 2 Month	After 3 Month	After 6 Month
Mean Size (in mm <sup>2</sup> )	48.38	46.22	44.62	42.17	40.36	38.8	36.9
S.D.	21.08	20.67	19.96	19.59	19.19	18.57	18.41

**Table 6: Comparison of Mean Size in mm<sup>2</sup> in Group B (Apthous ulcer) after Day 0, Day 1, Day 2**

Groups Comparison		Mean Difference	P value
Group B	Group C	-.06667	0.923(>0.05) (NS)
	Group D	-1.06667*	0.000 (<0.001) (HS)
Group C	Group B	.06667	0.923(>0.05) (NS)
	Group D	-1.00000*	0.000 (<0.001) (HS)
Group D	Group B	1.06667*	0.000(<0.001) (HS)
	Group C	1.00000*	0.000(<0.001) (HS)

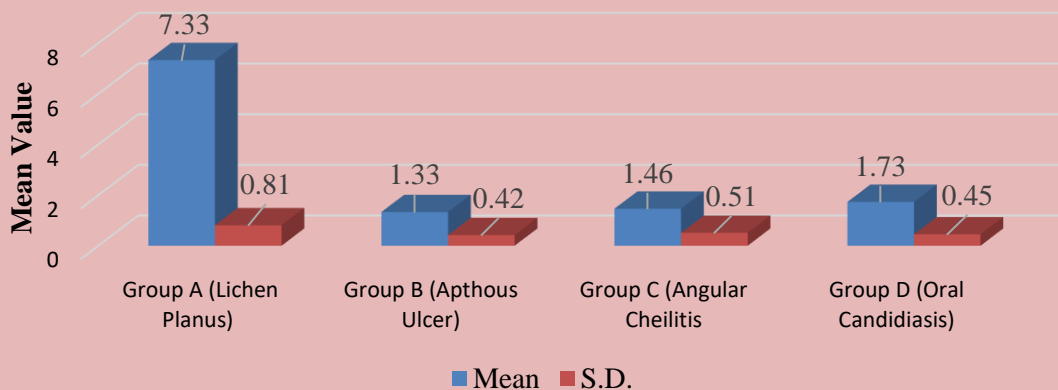
**Table 7: Comparison of Mean Size in mm<sup>2</sup> in Group C (Angular Cheilitis) after Day 0, Day 1, Day 2**

Group C	On Day 0	After 1 Days	After 2 Days
Mean Size (in mm <sup>2</sup> )	6.41	3.25	2.09
S.D.	1.76	1.39	0.67

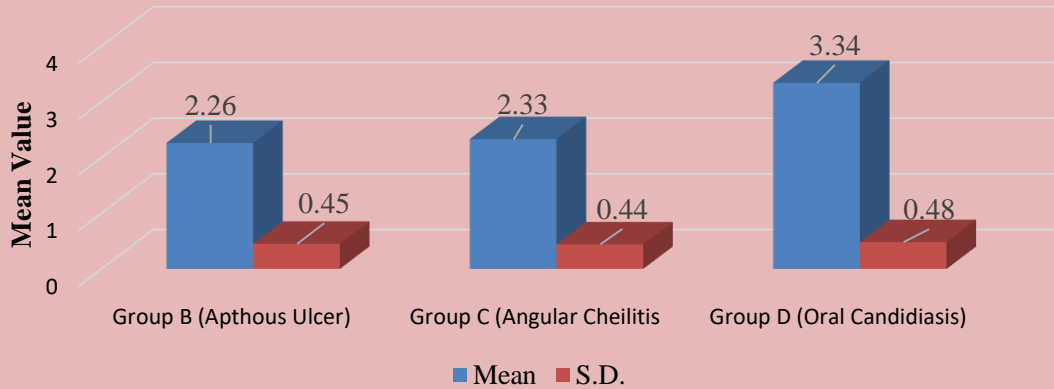
**Table 8: Comparison of Mean Size in mm<sup>2</sup> in Group D (Oral Candidiasis) after Day 0, Day 1, Day 2, Day 3**

Group D	On Day 0	After 1 Days	After 2 Days	After 3 Days
Mean Size (in mm <sup>2</sup> )	25.1	15.2	8.98	8.12
S.D.	4.87	5.3	4.8	2.64

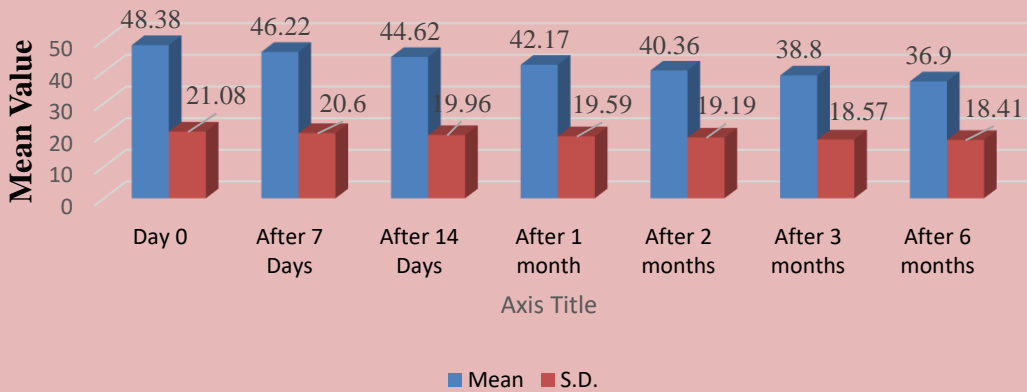
**Graph 1: Comparison of elimination of burning sensation after applying ozonized olive oil among all test groups**



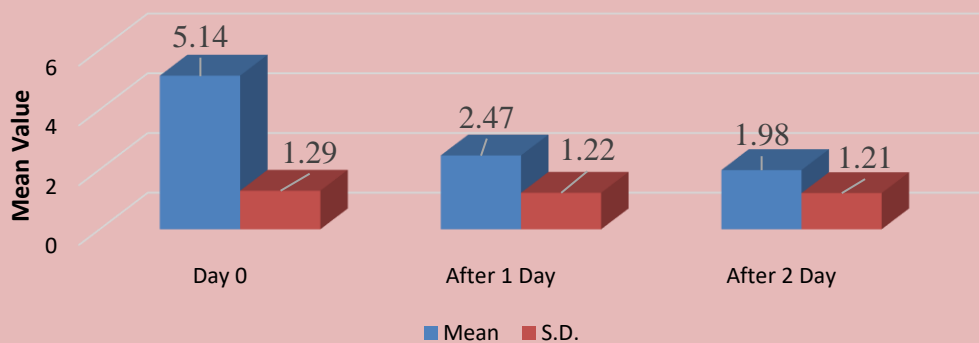
**Graph 2: Comparison of Mean days of complete healing after applying ozonized oil among all test groups**



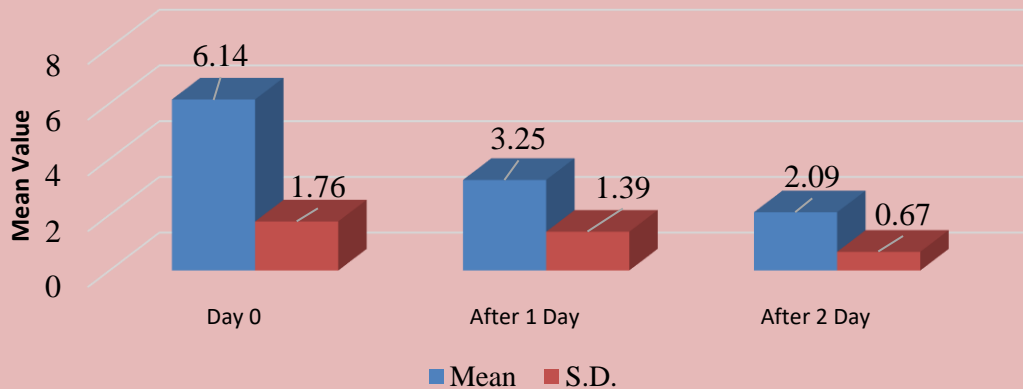
**Graph 3: Comparison of Mean Size in mm<sup>2</sup> in Group A (Oral Lichen Planus) Lesions after 0 day, 7 day, 14 days, 1 month, 2 months, 3 months, 6 months**



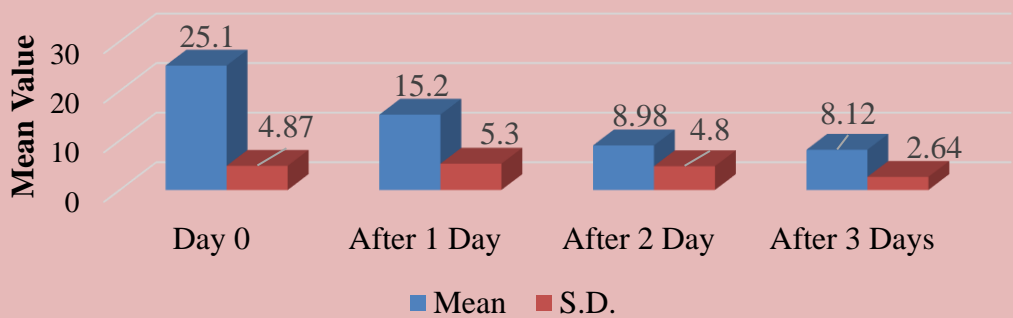
**Graph 4: Comparison of Mean Size in mm<sup>2</sup> in Group B (Apthous ulcer) after Day 0, Day 1, Day 2**



**Graph 5: Comparison of Mean Size in mm<sup>2</sup> in Group C (Angular Cheilitis) after Day 0, Day 1, Day 2**



**Graph 6: Comparison of Mean Size in mm<sup>2</sup> in Group D (Oral Candidiasis) after Day 0, Day 1, Day 2, Day 3**



**GROUP A – ORAL LICHEN PLANUS**

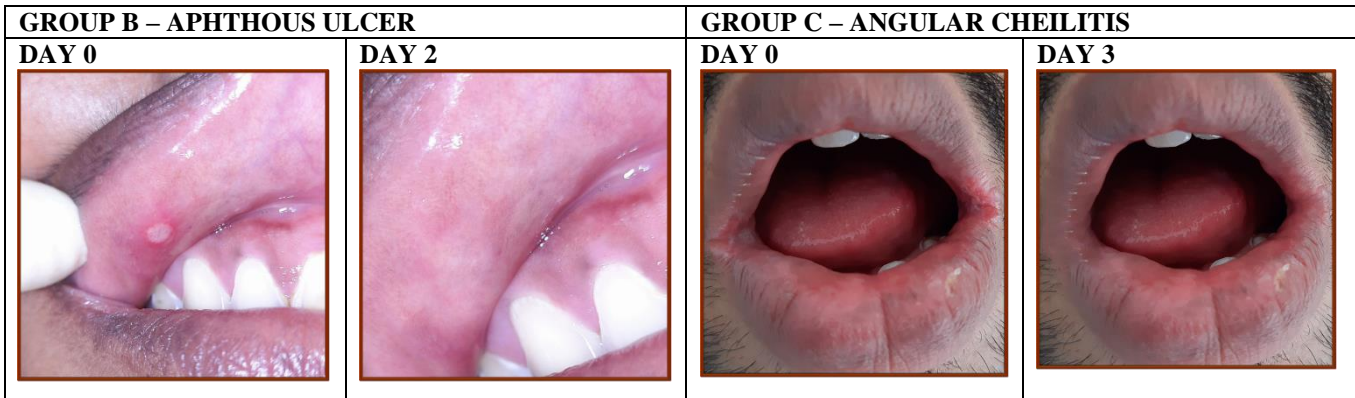
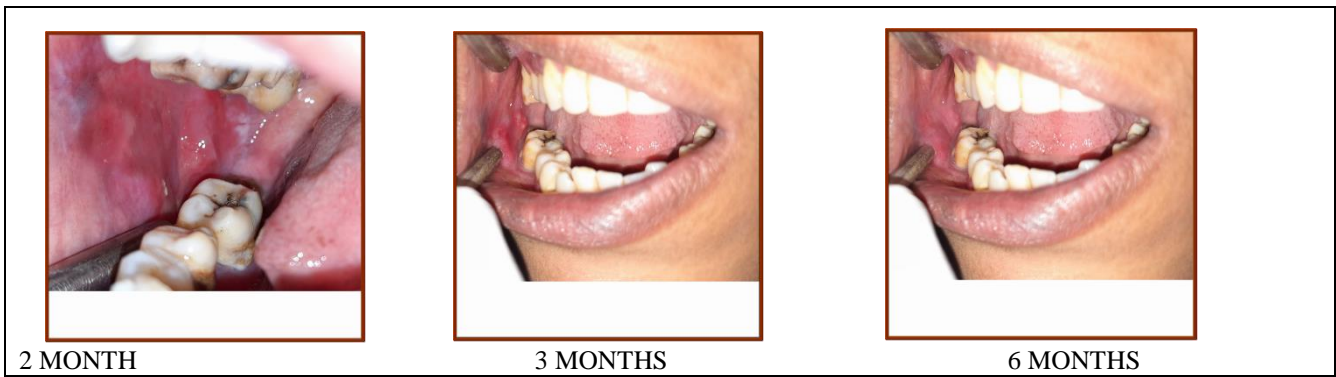


DAY 0

DAY 7

DAY 14

1 MONTH



**DISCUSSION**

Oral mucosal lesions are very common and we encounter them frequently in our day to day practice. There are various types of lesions like white lesions, red lesions, ulcerative lesions, vesiculobullous lesions etc. All lesions present with different tissue surface appearance such as white lesions may appear as reticular, plaque-like, popular or pseudomembranous, which affect the clinical appearance of the lesions. Red lesions may display a change of surface texture, which may become granular, velvety and rough.

Vesiculobullous diseases is a type of mucocutaneous diseases characterized by vesicles and bullae (i.e. blisters).

Recurrent aphthous ulceration is a common mucosal disorder that can be painful and debilitating for patients; has no known etiology and has a variety of

clinical manifestations ranging from single to multiple ulcers affecting the oral mucosa.

Angular cheilitis is a condition that caused red, swollen patches in the corners of the mouth where lips meet and make an angle. It can be unilateral and bilateral both. Main causes are saliva accumulation in the corner of the mouth, vitamin B12 deficiency, fungal infections (candida) etc.

Oral lichen planus (OLP) is a chronic inflammatory disorder of the oral mucosa of unknown etiology, affecting approximately 2% of the population. The pathogenic mechanism consists of apoptosis of basal keratinocytes induced by CD8+ T cell because of an underlying immune disorder. Typically the disease presents with multiple lesions, mostly with bilateral and symmetric distribution.

Candidiasis is a primary and secondary infection which is caused by *Candida* species especially *Candida albicans*. Its clinical symptoms are acute, sub-acute or chronic and sporadic. Infection may be limited to mouth, throat, skin, vagina, fingers, nails, trachea, lung or digestive system.

There are various treatment modalities used over the years to treat such oral mucosal lesions such as corticosteroids, retinoid, calcineurin inhibitors, laser, photodynamic therapy etc. Now a days there are various types of research carried out about other possible treatment modalities of oral mucosal lesions and among them topical application of ozone therapy has shown quite promising result & it is highly beneficial and effective in oral mucosal lesions.

Ozone therapy has gained a prominent consideration in the medical & dental fields due to its physicochemical properties. It possess strong antimicrobial activity against bacteria, viruses, yeasts and protozoa; a powerful oxidizing agent, anti-hypoxic, immunostimulating and anti-inflammatory. Ozone acts on bacterial cell membranes by oxidizing lipid & lipoprotein cell components, causing the deterioration of the internal bacterial membrane. Ozone application in the form of oil is used due to its cost effectiveness, easy manipulation and better shelf life.

In our study 60 patients selected which were divided into four group Group A (Oral lichen planus), Group B (Aphthous ulcer), Group C (Angular cheilitis) & Group D (Oral candidiasis) and each group consist of 15 patients. Comparison of elimination of burning sensation after applying ozonized olive oil among all test groups done and we found Mean days of elimination of burning sensation of Group A (Oral lichen Planus) patients were 7.33 with a standard deviation of 0.81. In conjunction to our study, Kumar T et al. (2016) in their study found mean days for elimination of burning sensation of lichen planus patients were 4.6 days. This assertion is too some extend similar to our study. Exposure to ozone results in a change in the level of a variety of biological factors (cytokines, interferon c, tumor necrosis factors a, transforming growth factors b & interlukin-8) which leads to reduce the sign and symptoms in OLP patients.

El Meligy M A et al. (2022) in their study found that elimination of burning sensation in erosive/atrophic lichen planus patient (10 patients) took around 3 months of time period which is not similar to our study. In this study they found the significant reduction in TNF $\alpha$  value at 1 month but there was increase in TNF $\alpha$  value at 3 months, which took them longer time to reduce the sign and symptoms of the patients.

Mean days of elimination of burning sensation of Group B (Aphthous Ulcer) patients were 1.33 with a standard deviation of 0.42. In conjunction to our study, Kumar T et al. (2016) in their study found mean days of elimination of burning sensation of recurrent aphthous stomatitis were 1.5 days; which is similar to our study.

There is increased motility and adhesion of peripheral blood PMN cells to epithelial cell lines after exposing to ozone. Which leads to fast healing in aphthous ulcer patients

K.AL-Omiri M et al. (2016) in their study found that elimination of pain/burning sensation in RAS patient took around 7-10 days, which is slightly higher in comparison to our study result. This can be explained by the fact that K.AL-Omiri et al. included both minor and major aphthous patients in which major aphthous ulcer patients took more time.

Mean days of elimination of burning sensation of Group C (Angular Cheilitis) patients were 1.46 with a standard deviation of 0.51. In conjunction to our study, Kumar T et al. in their study found mean days of elimination of burning sensation of angular cheilitis were 2.3 days; which was close to the result of our study.

Casu C et al. (2019) in their study found that elimination of burning sensation in recurrent exfoliative cheilitis patient took in 2 days, which is similar to our study. There is increased motility and adhesion of peripheral blood PMN cells to epithelial cell lines after exposing to ozone, which leads to fast healing in angular cheilitis patients.

Mean days of elimination of burning sensation of Group D (Oral Candidiasis) patients were 1.73 days with a standard deviation of 0.45. In conjunction to our study, Kumar T et al. (2016) in their study found mean days of elimination of burning sensation of candidiasis were 2.1 days; which was similar to the result of our study. Ozone has an antibacterial as well as antifungal activity. It helps in disruption of cell wall of the bacteria and inhibiting the protein synthesis during growth of microbes and finally breakdown it, which leads to better healing in oral candidiasis patients.

In our study we have done multiple pairwise comparison of mean days of burning sensation elimination after Ozonized olive oil application and we found that as compared to Group A (OLP) patients; Group B (Aphthous ulcer) patients shows fast elimination of burning sensation. The mean difference was 6.00000. Kumar T et al. (2016) in their study found the mean difference was 3.1; which was close to the result of our study.

As compared to Group B (Aphthous ulcer) patients; Group C (Angular cheilitis) patients shows delayed elimination of burning sensation. The mean difference was -.13333. Kumar T et al. (2016) in their study found the mean difference was -.8; which was close to the result of our study.

As compared to Group C (Angular cheilitis) patients; Group D (Oral candidiasis) patients shows delayed elimination of burning sensation. The mean difference was -.26667. Kumar T et al. (2016) in their study found the mean difference was 0.2; which was not similar to the result of our study.



As compared to Group A (OLP) patients; Group D (Oral candidiasis) patients shows fast elimination of burning sensation. The mean difference was 5.60000. Kumar T et al. (2016) in their study found the mean difference was 2.5; which was close to the result of our study.

As compared to Group A (OLP) patients; Group C (Angular cheilitis) patients shows fast elimination of burning sensation. The mean difference was 5.86667. Kumar T et al. (2016) in their study found the mean difference was 2.3; which was close to the result of our study.

As compared to Group B (Apthous ulcer) patients; Group D (Oral candidiasis) patients shows delayed elimination of burning sensation. The mean difference was -.40000. Kumar T et al. (2016) in their study found the mean difference was -0.6; which was close to the result of our study.

In our study we have done the comparison of Mean days of complete healing after applying ozonized olive oil among all test group and we found that mean days of complete healing in Group B (Apthous Ulcer) patients were 2.26 with a standard deviation of 0.45. Kumar T et al. (2016) in their study found mean days of complete healing of apthous ulcer patients were 1.5; which is close to the result of our study.

K.AL-Omiri et al. (2016) in their study found mean days complete healing of apthous ulcer patients were 10; which is not supported our study result. As they took major and minor apthous ulcer both. In case of major ulcers it took longer time to heal.

Logan R et al. (2005) in their study found that after topical ozone therapy complete healing of a major apthous ulcer on lateral border of tongue took approx. 1 month of time; which is not similar to our study. Here previously the patient was on corticosteroid therapy and there was not much success and later treatment modality switched to ozone so it took some more time to heal.

In our study mean days of complete healing in Group C (Angular Cheilitis) patients were 2.33 with a standard deviation of 0.44. Kumar T et al. (2016) in their study found mean days of complete healing of angular cheilitis patients were 2.3; which is supported by our study.

Casu C et al. (2016) in their study found that complete healing of angular cheilitis patient took 2 days; which is close to the result of our study.

In our study mean days of complete healing in Group D (Oral Candidiasis) patients were 3.34 with a standard deviation of 0.48. Kumar T et al. (2016) in their study found mean days of complete healing of candidiasis patients were 2.1; which is close to the result of our study.

In our study Multiple Pairwise Comparison of Mean Days of healing among all the groups done and we found; as compared to Group B (Apthous ulcers) patients;

Group C (Angular cheilitis) patients showed delayed healing. Kumar T et al. in their study found Group B (Apthous ulcer) patients healed a little faster than Group C (Angular Cheilitis) patients; which is similar to our study.

As compared to Group C (Angular cheilitis) patients; Group D (Oral candidiasis) patients shows delayed healing. Kumar T et al. in their study found Group D (oral candidiasis) patients healed a little faster than Group C (Angular Cheilitis) patients; which is not supported by our study.

Group B (Apthous ulcers) patients; Group D (Oral candidiasis) patients showed delayed healing. Kumar T et al. in their study found Group B (Apthous ulcer) patients healed a faster than Group D (Oral candidiasis) patients; which is similar to our study.

In our study comparison of mean size (mm<sup>2</sup>) in Group A (OLP) lesions on Day 0, Day 7, Day 14, 1 month, 2 month, 3 month and 6 month is 48.38, 46.22, 44.22, 42.17, 38.8 and 36.9 respectively.

Veneri F et al. in their study also found a difference in reduction of size of the lesion in 3 months of follow up, which supported our study.

In our study comparison of mean size (mm<sup>2</sup>) in Group B (Apthous ulcer) on Day 0, Day 1 and Day 2 is 5.14, 2.47 and 1.98 respectively.

K.L-Omiri M et al. (2016) in their study found reduction in size in apthous ulcer patient on Day 0, Day 1 and Day 2 is 5.20, 5.20 and 5.12 respectively. Which is not similar as our study result. As they took both major and minor apthous ulcer and in case of major apthous ulcer reduction in size was not that fast.

In our study comparison of mean size (mm<sup>2</sup>) in Group C (Angular cheilitis) lesions on Day 0, Day 1 and Day 2 is 6.41, 3.25 and 2.09 respectively. As per our knowledge still there is no such study conducted yet.

In our study comparison of mean size (mm<sup>2</sup>) in Group D (Oral candidiasis) lesions on Day 0, Day 1, Day 2 and Day 3 is 25.1, 15.2, 8.98 and 8.12 respectively. As per our knowledge still there is no such study conducted yet.

## SUMMARY & CONCLUSION

Oral mucosal lesions need proper diagnosis and accurate treatment plan for fast and better healing. A patient who has sustained with oral mucosal lesion undergoes through clinical examination as well as histopathological examination to establish the final diagnosis. Various treatment modalities used over the years to treat oral mucosal lesions such as topical and systemic corticosteroids, LLLT (Low level laser therapy), local anaesthetic gel application, retinoid, lycopene etc.

With the help of various researcher ozone therapy comes as a boon into the field of Medical as well as Dentistry. Ozone has been used in the field of medicine for over a century as a disinfectant, to purify blood and during the World War I it was used to treat

gangrene, burn wounds, osteomyelitis, fistulas and many other infections.

Ozone is known to be a potent oxidizer and has the ability to oxidize any known biological entity. The principal action of ozone is its antimicrobial effect on bacteria, virus and fungi, besides its Immunomodulatory, anti-hypoxic, biosynthetic and anti-inflammatory properties.

The present study showed 100% cure rates in aphthous ulcer, angular cheilitis, oral candidiasis patients and improvements in oral lichen planus patients can be due to above said mechanism. No patients in this study

showed adverse effects or toxicity, showing the safety margins of topical agents.

Ozone therapy is bound to revolutionize the way dentistry is going to be practiced in the future. The use of ozone to treat oral and dental diseases, indeed, is a paradigm shift in clinical practice.

Within the limitation of the study of a small number of cases, the healing of the lesions showed faster rates compared to the other conventional treatments, depicting the higher efficacy of the topical ozone therapy. So, a multicentre study with larger sample size is invited.

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