RESEARCH ARTICLE

# Evaluation of Salivary Lactate Dehydrogenase in Oral Submucous Fibrosis Patients Using Topical Triamcinolone Acetonide Gel with and without Iontophoresis: A Randomized Preliminary Study

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## Abstract

**Aim:** To use iontophoresis in conjunction with triamcinolone acetonide (TA) in ameliorating the symptoms of oral submucous fibrosis (OSMF) and further to evaluate the levels of the salivary lactate dehydrogenase (LDH) enzyme pre- and post-intervention.

**Materials and methods:** Forty-five OSMF patients were divided equally into 3 groups with 15 patients each. Salivary LDH levels of these 45 OSMF patients were assessed and compared with those of healthy controls. Comparison of pre- and post-treatment salivary LDH levels was also done.

**Results:** The salivary LDH level of OSMF patients (517.63 ± 150.80 U/L) was higher than that of the controls (141.70 ± 49.98 U/L). All the patients showed a significant ($p > 0.001$) decrease in salivary LDH levels following treatment, with a maximum reduction seen in group III.

**Conclusion:** Group III had shown better results than group I and II with a maximum decline in LDH levels in group III.

**Clinical significance:** In OSMF, TA, an intermediate-acting steroid, is commonly used by practitioners in the form of topical gels and intralesional injections; however, the drawback with the topical application is the wash-away of the drug through saliva while intralesional injections have the disadvantage of pain and discomfort due to multiple punctures, invasiveness, less patient acceptability, and chances of post-treatment fibrosis. To overcome such barriers, "iontophoresis", an electromedical method of transmucosal drug delivery to a specific anatomical site, could serve as a surrogate means that does not require venipuncture (an invasive technique). Estimation of LDH levels in saliva as a manifestation of cellular necrosis and oxidative stress can serve as a precise indicator of lesions disturbing the integrity of the oral mucosa.

**Keywords:** Iontophoresis, Lactate dehydrogenase, Oral submucous fibrosis, Triamcinolone acetonide.

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## Introduction

Oral submucous fibrosis (OSMF) is a chronic, insidious, and disabling condition. The approval and commercialization of tobacco and areca nut produces have been linked to a sudden upsurge in the rate of occurrence of OSMF.

Management of OSMF has also been tried by using topical steroid TA, which acts as an immunosuppressant, anti-inflammatory, and antifibrotic agent. Oral transmucosal drug delivery (OTDD) of TA can be reinforced by iontophoresis in which externally applied electric potential augments the flux of ionic compounds through membranes. Iontophoresis also serves as a therapeutic routine with an advantage of superior patient compliance.[1]

Lactate dehydrogenase (LDH) is a key salivary biomarker that could aid in the diagnosis of pathologic processes and may further play a significant role in habit cessation. However, there are inadequate data in the literature to support the association of OSMF with the LDH enzyme and further to serve as an essential biomarker in saliva.[2]

The LDH is a predominant enzyme that was learned in the ancient era of enzymology. Successively, LDH levels in saliva as an expression of cellular necrosis and oxidative stress can be considered as a precise indicator of lesions disturbing the integrity of the oral mucosa. There is a paucity of studies investigating the salivary LDH either as total or isoenzymes in oral cancer or OSMF patients.[3]

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As there is a direct relationship between glycolytic activity and malignant transformation of the lesion, the biochemical estimation of LDH activity is believed to be an early indicator of malignancy. It helps in providing presumptive evidence of the possibility of cancer.

However, few studies have compared serum LDH activity in oral malignancies and in premalignancies and their efficacy in predicting the behavior and progression. With the progress in dysplastic changes, there is always a tendency toward increased utilization of an anaerobic phase of the glycolytic pathway leading to a surge in serum LDH level, which is the basic enzyme utilized in the process. These metabolic modifications facilitating a high rate of glycolysis occur at an early stage of advancing carcinogenesis,

when the development of morphological malignancy may still be one of several possible endpoints.[4]

To the best of our knowledge, iontophoresis in conjunction with TA has not been tried in ameliorating the symptoms of OSMF, so this present novel study has been planned to evaluate the levels of the salivary LDH enzyme pre- and post-intervention.

## Materials and Methods

### Objective

To evaluate and compare salivary LDH levels in OSMF patients before and after treatment with topical TA, iontophoretic TA, and a combination of both.

### Source of Data

The present clinical study was carried out in the Department of Oral Medicine and Radiology, College of Dental Sciences, Davangere. The study was approved by the institutional review board (IRB). Patients attending the outpatient department (OPD) were examined and selected after obtaining informed consent. A total of 45 OSMF patients of either sex were enrolled in the study based on the following inclusion and exclusion criteria:

### Inclusion Criteria

- Patients clinically diagnosed with OSMF.
- Patients who agreed to quit the habit.
- Patients who had not received any prior treatment for OSMF.
- Patients with a mouth opening >20 mm.
- Patients who would comply with regular follow-up visits.

### Exclusion Criteria

- OSMF patients with pericoronal infection and temporomandibular joint (TMJ) dysfunctions.
- OSMF patients with coexisting systemic illness.
- OSMF patients with coexisting oral lesions.

### Study Groups

- Salivary LDH level was measured in 20 apparently healthy individuals who did not have any oral lesions and adverse habits.
- Salivary LDH level was also measured in all 45 patients before and after the treatment.

### Gel Preparation and Procedure of Drug Application[5]

The TA topical and iontophoretic gel was prepared at Bapuji Pharmacy College, Davangere. The topical 0.1% TA gel was applied by the patient themselves on the right and left buccal mucosa and lower labial mucosa for a total period of 6 weeks after meals in a tapering manner thrice daily, twice daily, and once daily for 2 weeks each. The patients were instructed to press a small dab (about 6 mm) to the lesion until a thin film develops and to avoid rubbing of the gel. They were instructed not to eat or drink fluids for half an hour after the application of the gel. The 0.1% iontophoretic gel was delivered to the patient by the investigator using a negative electrode (i.e., cathode) carrying the same charge as the drug, and the ground electrode of opposite charge (positive electrode, i.e., anode) was placed elsewhere on the body (palm of the patient and instructed to hold it firmly) to complete the circuit. Autoclavable electrodes were used. For the affected area, the current knob was turned clockwise fully to preset the unit to the clinically indicated iontophoresis current of 1.5 to 1.8 mA, 9 V. The cathode was rolled over the right and left buccal and lower labial mucosa. The duration of application of the iontophoretic gel by the electrodes was 25 minutes (10 minutes each for the buccal mucosa and 5 minutes for the lower labial mucosa) for each patient once a week for a total period of 6 weeks. The upper labial mucosa being difficult to retract fully was not included for iontophoresis therapy owing to poor accessibility and visibility issues. Each patient enrolled in the study was given around 12 tubes of 0.1% TA gel containing 5 g per tube for the drug application during the intervention.

### Study Parameters

A total of 45 out of 52 OSMF patients who fulfilled the criteria were enrolled in the study. A structured proforma was used to collect relevant information from each patient. Before commencing the treatment, the patients were counseled to quit the habit and reevaluated after 1 week and enrolled in the study. They were randomly divided into 3 groups with 15 patients in each group.

Group I was administered topical TA 0.1% gel as described earlier.

Group II patients received TA gel by iontophoresis therapy application.

Group III received a combination of group I and group II—that is, the topical application of TA gel daily, along with iontophoresis once a week for a total of 6 weeks. The patients were instructed not to apply topical TA on the day of the iontophoresis application.

### Salivary LDH Estimation Method[6]

The method employed in our study was the UV kinetic method recommended by the International Federation of Clinical Chemistry (IFCC) and the 1994 recommendation of Deutsche Gesellschafts für Klinische Chemie (DGKC 1994). The samples were analyzed in the auto analyzer Cobas Integra 400 (serial number 38-2665) using the reagent kit.

### Reagents

Reagent 1 (coenzyme)

- NADH 240 µmol/L

Reagent 1A (buffer)

- Tris buffer pH 7.20 80 µmol/L
- Sodium chloride 200 µmol/L
- Pyruvate 1.6 µmol/L

### Procedure

The patients were asked to sit comfortably with their head in upright position; they were asked to rinse the oral cavity using 30 mL of normal water and then were asked to accumulate the saliva in their oral cavities (unstimulated whole saliva) for 5 minutes. The patients were asked to spit the accumulated saliva in a sterile, disposable container, until a minimum desired quantity of 2 mL was obtained. The saliva from each patient was collected and centrifuged. The saliva samples and the reconstituted reagent were maintained at room temperature prior to use. The saliva sample was transferred to sterile disposable vials in the vial rack of the Cobas Integra analyzer. Once programd, the analyzer automatically pipettes 20 µL of the sample which was added to 1 mL of the reconstituted reagent into another vial. This was followed by calculation of the salivary LDH activity of each sample, for a minimum of four readings at intervals

of 60 seconds, and the readings were noted down and expressed in units/liter at 370°C. The clinical parameters and salivary LDH levels obtained before and after treatment were statistically analyzed.

## Results

### Salivary LDH Level between Controls and Patients (Fig. 1)

The mean salivary LDH level for the controls was 141.70 ± 49.98 U/L and for all 45 patients at baseline was 517.63 ± 150.805 U/L, respectively. The difference between patients and controls was statistically highly significant ($p < 0.01$).

### Pre- and Post-treatment Comparison of Salivary LDH Levels (Table 1, Fig. 2)

The mean salivary LDH values for group I, II, and III before the commencement of treatment and at the end of treatment had shown a definite improvement which was statistically significant ($p < 0.05$). The mean salivary LDH values decreased in all three groups, with a maximum decrease being witnessed in group III pursued by group II and group I.

### Correlation of Salivary LDH with Clinical Staging and Levels before and after Treatment

Decrement in pre- to post-salivary LDH levels in different stages were statistically significant ($p < 0.05$), and there was a reduction in the values at each stage:

- Stage I—442.50 ± 137.89 U/L to 112.00 ± 43.08 U/L
- Stage II—493.86 ± 158.85 U/L to 132.68 ± 55.69 U/L
- Stage III—549.35 ± 162.17 U/L to 122.92 ± 24.61 U/L
- Stage IV—472.33 + 29.02 U/mL to Stage III

## Discussion

Oral submucous fibrosis is a chronic, progressive, and potentially malignant disorder of the oral cavity. Triamcinolone acetonide (TA) is an intermediate-acting steroid commonly used by practitioners in the form of topical gels and intralesional injections; however, the drawback with the topical application is the wash-away of the drug through saliva, while intralesional injections have the disadvantage of pain and discomfort due to multiple punctures, invasiveness, less patient acceptability, and chances of post-treatment fibrosis. To overcome such barriers, iontophoresis, an electromedical method of transmucosal drug delivery to a specific anatomical site, provides an alternative route that does not involve venipuncture (an invasive procedure). Estimation of LDH concentration in saliva as an expression of cellular necrosis can be considered to be a specific indicator of lesions affecting the integrity of the oral mucosa.

A statistically significant difference was detected between the LDH levels of controls and patients, which can be attributed to the upsurge of oxidative stress in OSMF patients.

The observation is in accordance with a study in which the LDH level for OSMF patients was more than that of the controls. The researchers observed that the average salivary LDH value for OSMF patients to be 606.83 ± 60.09 U/L and for healthy controls, it was 80.73 ± 20.06 U/L. Salivary LDH was found to be greater in OSMF patients than in healthy controls, and the difference was statistically significant. On comparing the serum and salivary LDH levels with the clinical staging of OSMF, the results were

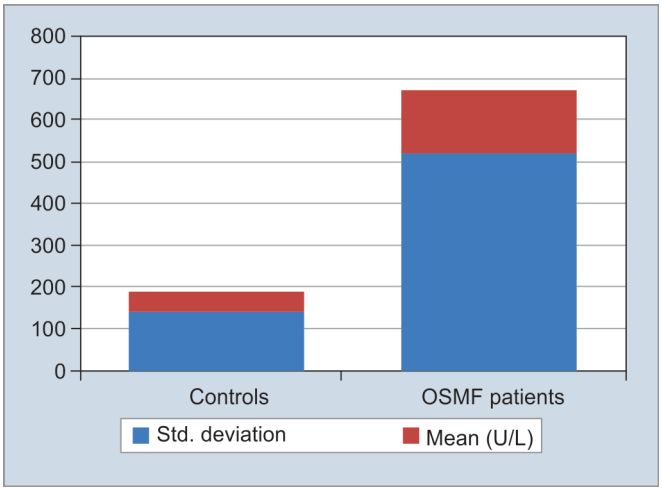


**Fig. 1:** Comparison of salivary LDH levels between the control and patients

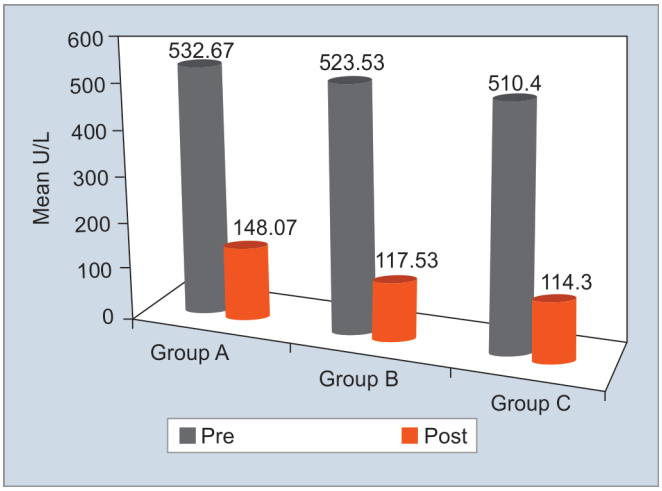


**Fig. 2:** Pre- and post-treatment LDH levels

**Table 1:** Comparison of salivary LDH levels pre- and post-treatment

| | | | | | | *ANOVA* | |
|---|---|---|---|---|---|---|---|
| *Salivary LDH* | | *Mean (U/L)* | *SD* | *Minimum* | *Maximum* | *F* | *p values* |
| Pre | I | 532.67 | 147.42 | 343 | 876 | 0.08 | 0.93 (NS) |
| | II | 523.53 | 161.50 | 292 | 850 | | |
| | III | 510.40 | 165.53 | 283 | 837 | | |
| Post | I | 148.07 | 50.27 | 78 | 215 | 2.57 | 0.09 (NS) |
| | II | 117.53 | 48.76 | 43 | 201 | | |
| | III | 114.33 | 34.21 | 59 | 187 | | |

not statistically significant. Similarly, they found no statistically significant relationship on comparing the serum and salivary LDH in OSMF subjects with duration of habit.[6]

Our results were supported by another study that assessed the LDH enzyme in 80 OSMF patients and 80 healthy controls. The results revealed a significant increase in the salivary LDH levels in OSMF patients when compared to normal healthy individuals ($p < 0.001$). A positive correlation was observed between those who had submucous fibrosis and with the duration of areca nut chewing.[2]

Another study was on a par with our study and reported a definite significant increase in the LDH levels in oral cancer and OSMF when compared to healthy controls (mean salivary LDH level in the HC, OSMF, and OC groups were 126.7 ± 58.2 IU/L, 612.2 ± 328.9 IU/L, and 515.7 ± 257.8 IU/L, respectively).[3]

The elevated salivary LDH levels may also be related to the hypoxic state seen in OSMF. Hypoxia triggers glycolytic pathways where the end product is a lactate. This reaction is mediated by the LDH enzyme. Thus, in these conditions, by reflex LDH levels are increased. The authors stated that increased hypoxia plays a role in malignant transformation and progression of OSMF. Although OSMF is a disease of connective tissue of the oral mucosa, there is alteration in the epithelium due to the abnormal changes occurring in the fibrous connective tissue. These altered epithelial cells might be the reason for the elevated salivary LDH levels in OSMF cases. Oral epithelial cells are the direct source of LDH in saliva rather than the salivary gland by itself.[2]

On correlating the salivary LDH level with clinical staging in our study, we found that the salivary LDH level was higher in stage III of OSMF and decreased with early stages of OSMF. Post-treatment levels of salivary LDH showed a direct correlation with staging as in stage I patients showed the least change in salivary LDH levels and maximal change in salivary LDH levels in stage III.

It was in accordance with the previously reported study wherein an increase in the LDH level was observed in 21 (26.25%) cases of grade I, 33 (41.25%) cases of grade II, and 26 (32.5%) cases of grade III severity of OSMF. There was a progressive increase in the enzyme levels with an increase in the severity of disease in saliva, which was found to be statistically significant ($p < 0.0001$).[2]

Overall correlation with the treatment outcome was not possible because of the unequal distribution of patients belonging to different stages. OSMF and its potential malignant transformation can be mainly attributed to fibrosis, hypoxia, muscle fatigue, and a shift to anaerobic glycolysis (i.e., the conversion of pyruvates to lactates). These conditions are usually associated with an increase in the LDH enzyme.[7] Pairwise comparison of salivary LDH levels between healthy control and the 3 groups of OSMF was statistically highly significant. A study conducted in 2015 on 60 volunteers reported a statistically significant correlation among various groups.[8]

Correlation of improvement in clinical parameters and salivary LDH levels clearly imply that a significant decrease in salivary LDH levels was attained in all three groups post-treatment.

To the best of our knowledge, until now no study has been conducted where the pre- and post-treatment levels of the salivary LDH enzyme have been measured following an intervention. So, at this stage, the present study cannot be compared with any other studies with reference to the LDH values before and after treatment.

## Conclusion

Iontophoresis in conjunction with TA was found to be effective in ameliorating the symptoms of OSMF and also showed a decrease in the levels of the salivary LDH enzyme pre- and post-intervention. Studies with a larger sample size and validity of salivary LDH levels with that of serum levels and further correlation of LDH levels with a subsequent development of malignancy are recommended.

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