

EFFICACY OF LYCOPENE AND SERRATIOPEPTIDASE IN THE MANAGEMENT OF ORAL SUBMUCOUS FIBROSIS: A PLACEBO-CONTROLLED FOLLOW-UP STUDY

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ABSTRACT

Introduction: Oral Submucous Fibrosis is a well-known potentially malignant disorder that is insidious and chronic in nature affecting the entire oral cavity, sometimes extending to the pharynx. Various conservative drug treatments and combination therapies that are currently in use are clearly effective and adequate. Hence the use of potential beneficial effects of antioxidants and enzyme therapies are becoming more popular. **Aim:** To evaluate the clinical efficacy of lycopene and serratiopeptidase in the treatment of oral submucous fibrosis for a follow-up period of four weeks. **Materials and Methods:** A total of 84 patients of oral submucous fibrosis were included, of which 30 patients were treated with Lycopene & Serratiopeptidase

each and 24 patients with placebo. The clinical examination like mouth opening, burning sensation, visual inspection and palpatory findings were evaluated on a weekly interval for a period of four weeks. **Results:** The evaluation of burning sensation, tongue protrusion, uvula involvement and palpatory findings showed significant improvement; whereas evaluation of mouth opening showed non-significant improvement in both Lycopene and Serratiopeptidase

group when compared with placebo. **Conclusion:** Based on the present results, it is possible to state that in addition to Lycopene, enzyme Serratiopeptidase have got a role in improving the signs and symptoms of oral submucous fibrosis. It is suggested that further studies on a larger sample size and for a longer follow up period were necessary to arrive at a broader approach.

KEYWORDS: Antioxidants; Burning Sensation; Enzyme Therapy; Free Radicals; Oxidative Stress.

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is a chronic, debilitating, insidious disease that is associated with significant functional morbidity.^[1] It is a well-recognized potentially malignant disorder of oral cavity which was first described by Pindborg and Sirsat.^[2] As stated in the article of Kerr *et al.*,^[3] Schwartz was the one who described oral submucous fibrosis in the tobacco-chewing women of Indian origin in East Africa. However Joshi *et al.*, were the first to describe this condition in India in 1952 and coined the term 'Oral Submucous Fibrosis'.^[4] OSMF initially affects the lamina propria of the oral mucosa and as the disease progresses it involves the submucosa and the deeper tissues including muscles of the oral cavity with resulting in loss of fibro-elasticity. The clinical manifestations include blanching and stiffening of the oral mucosa leading to limitation in oral opening along with burning sensation as a chief symptom.^[5]

Free radicals because of their role in various oxidation processes have emerged as mediators of phenotypic and genotypic changes that lead from mutation to neoplasia. A number of studies have proven that the management of premalignant lesions should include antioxidants along with the cessation of the habit.^[6,7] Keeping this as a key concern, a wide range of treatment modalities both medical and surgical have been proposed for OSMF, but none have proved curative or reduced the morbidity significantly.^[5] As a mode of newer treatment resolution, natural enzyme like Serratiopeptidase,^[8] and antioxidants like Lycopene,^[9] are found to be effective and are also being considered.

Serratiopeptidase or Serrapeptase is a proteolytic enzyme isolated from the non-pathogenic Enterobacteria *Serratia* E15 that has been successfully used for relieving pain and inflammation due to its primary anti-inflammatory action and has a fibrinolytic activity for resolving fibrin plaques and scars.^[8,10,11] Lycopene is a bright red carotenoid pigment and a phytochemical found in red fruits and vegetables such as tomatoes, carrots, watermelons and

papayas. Lycopene exerts most efficient antioxidant activity by physical and chemical quenching of free radicals such as singlet oxygen.^[12,13] In continuation of recognition of the need for the specific therapeutic regimen, the present study was conducted to evaluate the efficacy of Lycopene and Serratiopeptidase in Oral submucous fibrosis patients for a follow-up period of four weeks.

MATERIALS AND METHODS

The prospective experimental study included a total of 84 oral submucous fibrosis patients who presented to the out-patient Department of Oral Medicine and Radiology, Institute of Dental Sciences, Bareilly, during January till June 2017.

For establishment of oral submucous fibrosis diagnosis, two parameters mentioned below were to be satisfied for inclusion in the present study. (a) Positive history of chewing areca nut or one of its commercial preparations, difficulty in swallowing and chewing and burning sensation on eating spicy foods. (b) Restricted mouth opening and changes in the oral mucous membrane including the presence of palpable vertical fibrous bands, stiffness and blanching. The exclusion criteria included individuals with other associated oral mucosal lesions or systemic diseases, systemic conditions like pregnancy, lactating mother and those not willing for follow-up.

Detailed case history was documented and complete information about the study protocol, necessary follow-up required was explained and a signed informed consent was obtained from each patient. The procedures followed were in accordance with the ethical standards of the Institutional committee on human experimentation and with the Helsinki Declaration of 1975 as revised in 2008.^[14]

The group wise distributions of the sample size and the patients treated with Lycopene (LycoRed™) 8mg softgels, Serratiopeptidase (Seratid^R) 10mg tablets and Placebo (Jagsonpal Pharmaceuticals Ltd., New Delhi) are as mentioned in Table 1. Based upon the previous investigations,^[6,8,9] the present sample size was established. The sample size of 84 patients of OSMF was calculated by fixing Alpha at 5%, β error at 20%. So the power of the study was kept at 80% using G-Power.

Table 1: Group wise distribution of patients with OSMF being treated.

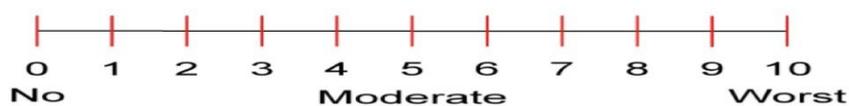
Study Groups	No. of Patients	Treatment	Dosagefor four weeks
Group A	30	Lycopene	16mg daily in 2 equally divided doses
Group B	30	Serratiopeptidase	30mg daily in 3 equally divided doses
Group C	24	Placebo	Twice daily
Total	84		

Following the establishment of the diagnosis, all the patients were advised to discontinue the associated habits. Patients were then evaluated and followed-up in a weekly interval for a period of four weeks. The clinical examination was carried out at every recall visit and the clinical findings were recorded with the parameters like mouth opening, burning sensation, visual inspection and the palpatory findings with excellent patient compliance.

Mouth Opening: The mouth opening was measured with a Vernier Caliper between the incisal tips of both the arches. The OSMF was graded clinically according to the classification of Ranganathan *et al.*^[15] into one of the four groups: (Table 2) **Burning Sensation:** Burning sensation was gauged based on Visual Analogue Scale ranging from 0-10. (Figure1).

Table 2: The clinically grading system of oral submucous fibrosis.

Group	Clinical findings
Group I	Only symptoms, with no demonstrable restriction of mouth opening
Group II	Limited mouth opening, 20mm or more
Group III	Mouth opening less than 20mm
Group IV	OSMF advanced with limited mouth opening. Precancerous or cancerous changes seen throughout the mucosa

**Figure 1: Visual Analogue Scale.**

Visual inspection: The involvement of the uvula was recorded as positive when it appeared as shrunken or deviated with or without blanching. Later it was graded on a weekly intervals basis as 0 if there was no change and as 1 if any improvement from the base line measurement was noted. The degree of protrusion of tongue was recorded in millimeters from the incisal edge of the lower teeth to the tip of tongue. (Figure2).

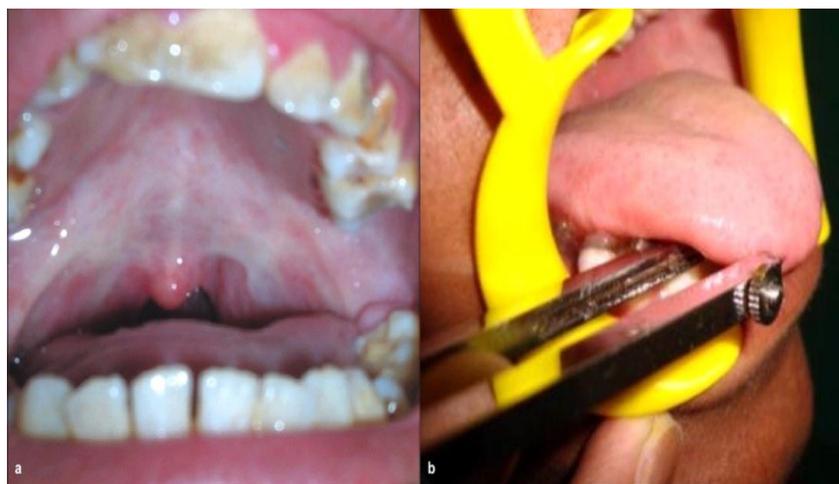


Figure 2: The clinical picture showing the assessment of uvula and tongue protrusion.

Palpatory findings: These were recorded as positive when a lack of suppleness and palpable fibrous bands or marked stiffness was evident for the areas of the right and left buccal mucosa, lips and floor of mouth. The site of the bands and its extent (including segmentation) in all the groups were recorded at the initial visit and later compared with weekly interval using the criteria: 1 as Improved, 0 no change and -1 as worsen.

Statistical analysis: All quantified variables thus measured, were recorded on the Microsoft excel software and subjected to statistical analysis using statistical package for social sciences (SPSS) software version 14. Analysis was carried out using Pearson's Chi Square test and Paired 't' test.

RESULTS

A total of 84 patients in which 76 were males and 8 were females with a age range of 18-62 years were noted. In the study population 90.5% of patients gave a positive history of Arecanut chewing either in the raw form, as a quid or in a commercial preparation such as Gutkha, which was the most common form used (76.2%). The rest 9.5% of patients had only tobacco chewing habit.

Evaluation of burning sensation among different groups showed no significant changes in 1st and 2nd week, but a highly significant ($P=0.001$) change were seen between the group treated with lycopene and placebo group in 3rd and 4th week and between seratid and placebo group in 4th week respectively.(Table 3).

Table 3: Statistical evaluation of burning sensation among the study groups.

Study Groups	1 week		2 week		3 week		4 week	
	Mean±SD	<i>p</i> -value	Mean±SD	<i>p</i> -value	Mean±SD	<i>p</i> -value	Mean±SD	<i>p</i> -value
A vs B	6.00±2.07	0.224	4.87±1.95	0.582	3.47±1.64	0.621	3.00±1.77	0.352
A vs C	6.00±2.07	0.401	4.87±1.95	0.541	3.47±1.64	0.001(HS)	1.73±1.33	0.001(HS)
B vs C	5.00±2.33	0.692	4.47±1.95	0.273	3.80±2.04	0.627	3.20±1.96	0.001(HS)

Evaluation of mouth opening upon weekly follow-up among the study groups showed non-significant results. (Table 4) Assessment of visual inspection in the form of uvula involvement (Table 5) & tongue protrusion (Table 6) as well as palpatory findings (Table 7) showed significant improvement in both Lycopene and Serratiopeptidase group.

Table 4: Statistical evaluation of mouth opening among the study groups.

Study Groups	1 st week		2 nd week		3 rd week		4 th week	
	Mean(cm)±SD	<i>P</i> -value						
A vs B	28.60±6.79	0.357	30.27±6.85	0.491	32.07±6.28	0.732	33.93±6.22	0.984
A vs C	28.60±6.79	0.702	30.27±6.85	0.864	32.85±6.28	0.431	33.93±6.22	0.162
B vs C	31.60±10.35	0.621	32.47±10.28	0.476	33.13±10.46	0.384	34.00±10.40	0.271

Table 5: Statistical evaluation of uvula involvement among the study subjects.

Study Groups	Mean±SD		t value	p value
	1 st Week	4 th Week		
Lycopene	0.33±0.47	1.00±0.00	-7.616	0.000
Seratiopeptidase	0.33±0.00	1.00±0.00	-7.616	0.000

Table 6: Statistical evaluation of tongue protrusion among the study subjects.

Study Groups	Mean (cm)±SD		t value	p value
	1 st Week	4 th Week		
Lycopene	9.20±0.96	11.53±1.16	-14.456	0.000
Seratiopeptidase	9.20±0.96	11.33±0.95	-20.451	0.000

Table 7: Statistical evaluation of palpatory findings among the study subjects.

Study Groups	Mean±SD		t value	p value
	1 st Week	4 th Week		
Lycopene	0.33±0.04	1.00±0.00	-7.616	0.000
Seratiopeptidase	0.30±0.46	1.00±0.00	-8.226	0.000

DISCUSSION

Treatment for oral submucous fibrosis is a challenge, as the pathogenesis of this disease is ambiguous. It is said that, once the disease has established, there is neither regression nor any

effective treatment. Subsequently, enhanced oral opening and respite of symptoms form the objective of OSMF treatment.^[16]

Most imperative phase of medical treatment for OSMF is the termination of dependency of eating arecanut, betel quid, auxiliary local irritants, spicy and hot food, alcohol and smoking.^[17] Characteristically, if the disease is noticed prior to progress of trismus, termination of the betel habit will frequently resolve the disease. Once trismus has established and disease is classified as mild to moderate, OSMF is irreversible. Subsequently, the objective of medical and surgical rehabilitation is to conserve oral function and constraint its advancement. Treatment at this phase is concentrated on reestablishing mandibular range of motion, oral cancer scrutiny, and termination of betel nut habit. Physical therapy combined with medical treatment is often utilized.^[18] The usual approach of medical treatment methods embrace injection of steroids,^[19] placental extract, Interferon- γ ,^[20] hyaluronidase,^[21,22] use of trypsin, collagenase,^[23] elastase, lactate dehydrogenase,^[24] serratiopeptidase and lycopene,^[6,9] with varying benefits.^[25]

Serratiopeptidase is a proteolytic enzyme, obtained from the microorganism *Serratia*, which is naturally existent in the intestine of silkworms. This enzyme assists the budding moth to thaw its fibrous cocoon.^[26] It is having mechanism of action of anti-inflammatory by preventing the hydrolysis of bradykinin, histamine and serotonin which results in vasodilatation and secondly of fibrinolytic activity where by prevents the role of plasmin in the conversion of procollagen to collagen.^[10] Mazzone *et al.*, evaluated the efficacy of Serratiopeptidase in a multicenter, double-blind, placebo-controlled study of 193 subjects suffering from acute or chronic ear, nose or throat disorders. After 3-4 days' treatment, significant symptom regression was observed in Serratiopeptidase treated patients. Statistical comparison confirmed the greater efficacy of Serratiopeptidase against all the symptoms examined. It was concluded that Serratiopeptidase has anti-inflammatory, anti-edemic and fibrinolytic activity and acts rapidly on localized inflammation.^[27] Thus based upon the previous studies, a proposed mechanism of action of Serratiopeptidase in the treatment of oral submucous fibrosis has been formulated.(Figure 3).

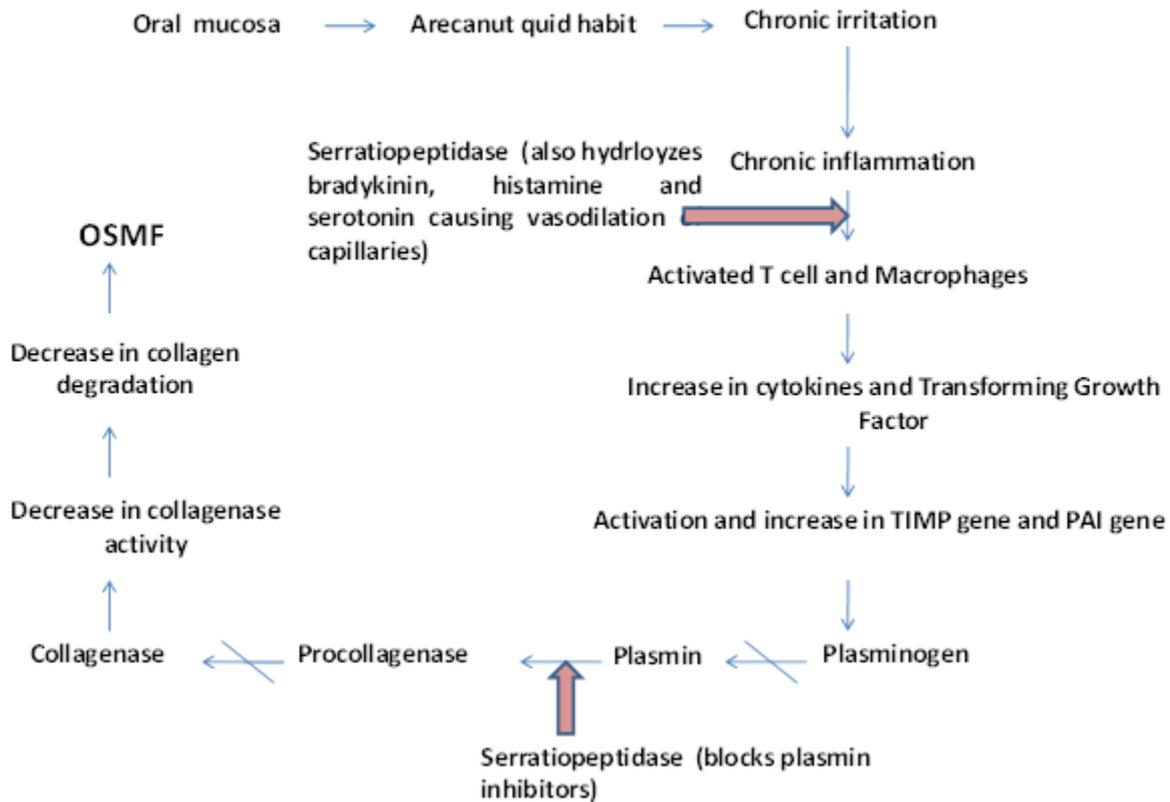


Figure 3: Proposed mechanism of action of Serratiopeptidase in the treatment of oral submucous fibrosis.

Carotenoids are biological colorants manufactured by plants and are accountable for the colors of vegetables and fruits. Lycopene is the carotenoid that contributes tomato its vivid red color, and it is one of the major carotenoids in diets. It reports for 50% of the carotenoids in human serum.^[28,29] Lycopene as an antioxidant has various benefits, such as interference with growth factor stimulation, inducing phase II enzymes, regulation of transcription, restoration of gap junctions and inhibition of cancer cell proliferation,^[30] as well as several other beneficial properties. Lycopene has revealed its effectiveness as a chemopreventive agent for oral premalignant lesions.^[31] Lycopene has been shown to inhibit hepatic fibrogenesis by Haque *et al.*,^[32] and more specifically in LEC rats by Kitade *et al.*^[33] which may thus exert a similar inhibition on abnormal fibroblasts in submucous fibrosis.^[6] Heber D *et al.*,^[34] demonstrated the action of Lycopene which upregulates lymphocyte resistance to stress and suppresses the inflammatory response.

In the present study, burning sensation of Lycopene and Serratiopeptidase groups showed statistically highly significant improvement. The results were in accordance with the study

done by Abhinav *et al.*,^[6] who stated that the post treatment burning sensation values showed improved results upon treating with lycopene.

In the present study improvement in mouth opening by both Lycopene and serratiopeptidase was statistically non-significant. However a contrasting results was noted in the Lycopene group by Abhinav *et al.*,^[6] and Bhagwan G *et al.*,^[9] who noted a statistically significant improvement in mouth opening. Since no other study has assessed the role of serratiopeptidase in OSMF in the literature, the results could not be compared directly with any other study.

Borle RM and Borle SR, treated 326 OSMF patients where one group received systemic anti-oxidants and another received submucosal triamcinolone injections. They noted that the patients treated with antioxidants showed better overall improvement and patients having relapse was lower than those treated with triamcinolone injections.^[35] Ameer NT *et al.*, showed a significant effect of triamcinolone in the treatment of oral submucous fibrosis and its effect depends on the stage of presentation and on the frequency of the chewing habits and not on the duration of the habits. They also concluded a highly significant improvement in inter-incisal mouth opening following treatment as compared to pre-treatment mouth opening.^[22]

Gupta S, *et al.*, evaluated Lipid peroxidation product, malonaldehyde (MDA) and antioxidants in plasma and erythrocytes of 34 cases of OSMF. While plasma MDA was found to be significantly higher in patients as compared to controls, plasma beta carotene and vitamin E levels were found to be decreased significantly in patients with respect to healthy controls. After 6 weeks of oral administration of beta-carotene and vitamin E, patients showed increase in plasma level of these two antioxidants along with decrease in MDA level associated with clinical improvement.^[36]

Irrespective of this study's findings, the potential benefits of Lycopene and Serratiopeptidase screening are enormous. However, based on the results of the present study, besides Lycopene, enzyme Serratiopeptidase also have a role in improving the signs and symptoms in OSMF patients.

LIMITATION/CONCLUSION

As the supporting literature is scarce on Serratiopeptidase for managing oral submucous fibrosis, larger trials with longer follow-up periods are needed to establish a definitive relationship. Longitudinal prospective multicenter studies using validated objective and subjective measures are required to further explore the use of lycopene, Serratiopeptidase or other novel therapies, for the treatment of OSMF.

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