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Research Article

CURCUMIN MUCOADHESIVE GEL IN MANAGEMENT OF OSMF: A CLINICAL AND HISTOPATHOLOGICAL STUDY

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A B S T R A C T

Introduction: Oral submucous fibrosis (OSMF) is a potentially malignant disorder carrying a high risk of malignant transformation. A wide range of treatment modalities have been proposed for oral submucous fibrosis but none have proved curative or reduced the morbidity significantly. Very few researches have shown the efficacy of curcumin as targeted local drug delivery in oral submucous fibrosis. Hence the study was planned. In vitro & in vivo studies suggested curcumin as an anticancer, antioxidant & anti-inflammatory agent. Thus based on this literature survey the study was undertaken.

Aim: To evaluate the efficacy of 10% curcumin mucoadhesive gel for the treatment of clinical stage 2 OSMF patients.

Study design & Sample size: This is an in-vivo single arm clinical study. The study sample included a total of 50 clinical stage 2 OSMF patients with clinically & histopathologically confirmed diagnosis.

Materials & methods: Patients were given 10% curcumin mucoadhesive gel and were instructed to apply topically two times per day making a daily dose of 1 gram. The primary outcome measures were to note the subjective symptoms and objective parameters. Subjective & objective parameters were entered as scores in the proforma. All measurements were taken by the same examiner to avoid observer variability. These parameters were analyzed at baseline, 15th day, 30th day, 45th day, 60th day & 75th day, 90th day, 4th month, 5th month and 6th month. Patients were also evaluated histopathologically after 6months.

Statistical analysis: The data collected were tabulated and analysed. The difference in scores at 15th day, 30th day, 45th day, 60th day, 75th day, 90th day, 4th month, 5th month and 6th month were compared by paired t test. 'p' value of 0.05 or less was utilized for statistical significance.

Results: Patients showed statistically significant improvement in all the subjective signs & symptoms, clinical staging & histopathological grading with p value of < 0.05.

Conclusion: It is evident from the study that curcumin holds good promise in the treatment of OSMF.

INTRODUCTION

Oral submucous fibrosis (OSMF) has been described as "an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although, occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by a fibro-elastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat."[1] Many treatment modalities in current practice for OSMF are circumstantial and most of the studies which tested various therapies lacked good design and planning. Hence, need of a good research and awareness still pertains to clinicians as well as patients [2].

Polyphenols play an important role in the maintenance of health and prevention of diseases. Among polyphenols, the most widely used substance is Curcumin. Curcumin is considered as safe, non-toxic and effective alternative for many traditional drugs because of its effects on various systems and therapeutic properties. Local drug delivery may provide a more targeted and efficient drug-delivery option than systemic delivery for diseases of the oral mucosa. Oral diseases can be effectively treated by local therapeutic approaches, due to the ease of the oral cavity accessibility [3].

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Shankar and co authors have reviewed the most common mucosal diseases and identified the current treatment approaches systemically and locally. In addition, they identified the novel biological therapies such as macromolecular biological drugs, peptides and gene therapy which may be of value in the treatment of many chronic oral conditions and if their delivery can be optimized [3].

Oral mucosa is a highly diverse, dynamic and responsive environment that despite high accessibility presents a number of challenges for oral drug delivery [4]. As a route of administration, it shows several advantages, where drugs are self-administrable and well accepted by patients. The oral mucosa is easily accessible and rapidly repairs itself after damage or trauma. This short recovery time limits potential adverse side effects caused by long-term topical drug delivery. In addition, there are fewer Langerhans cells in the oral mucosa than the skin reducing the risk of an allergic response [3]. Oral mucosal delivery has the potential to treat many different conditions and diseases. Each therapy requires distinct penetration and drug retention profiles in order to optimize treatment and minimize side effects [3].Very few researches have shown the efficacy of curcumin as targeted local drug delivery in oral submucous fibrosis. Hence the study was planned.

Aims and Objectives

The aim of the study was to evaluate the efficacy of 10% curcumin mucoadhesive gel in stage 2 OSMF patients.

The objective was to check the treatment efficacy of curcumin

- In terms of alleviating subjective symptoms like burning sensation, difficulty in mouth opening, intolerance to spicy food and difficulty in swallowing, signs like shrunken uvula, hockey stick appearance of uvula, and objective parameters like blanching, sites of fibrosis, burning sensation, pain, mouth opening, tongue protrusion & cheek flexibility.
- Post treatment changes in clinical staging
- Post treatment histopathological changes and grading

METHODOLOGY

Study design

This is an in-vivo single arm clinical study, conducted at HKE'S S.N institute of dental sciences & research center Gulbarga. Informed consent was obtained from all the subjects who were included in the present study. The study was approved by Institutional Ethical Committee, Dental College and Hospital as per Rajiv Gandhi University of Health Sciences, Karnataka, India (ECM/HKES/SNDCH/2012-2013) and was registered for clinical trials in the 'US clinical trials registry' (ClinicalTrials.gov; ref no. (NCT03511261).

Study samples & Sample size

OSMF patients from the department of oral medicine and radiology from HKE'S S.N institute of dental sciences & research center and Al-Badar rural dental college & hospital were selected by simple random sampling technique. The study sample included a total of 50 clinical stage 2 OSMF patients with clinically & histopathologically confirmed diagnosis.

Inclusion criteria

50 clinical stage 2 OSMF patients selected randomly with clinically & histopathologically confirmed diagnosis

Exclusion criteria

Clinical stage 1 & 3 OSMF patients, Patients underwent/undergoing treatment for OSMF. Patients allergic to curcumin, clinically diagnosed cases not ready for incisional biopsy, patients suffering from medically compromised conditions.

Procedure

The study was conducted by strictly adhering to the ethical protocols. Patient's personal history of habits was recorded. Diagnosis of OSMF was done by the criteria given by Bailoor D.N & Nagesh (2005) [5] for presence of burning sensation, blanching of the oral mucosa, restricted mouth opening, restricted tongue protrusion & palpable fibrous bands. Clinical staging of OSMF was done according to Mathur & Jha [6], Bailoor & Nagesh [7]**.** Clinical stage 2 cases were included in the study. Patients were encouraged for habit cessation & were subjected to oral prophylaxis to motivate them for abstinence. Symptoms like burning sensation, difficulty in mouth opening, difficulty in swallowing, intolerance to spicy food was noted. After 1 month of discontinuation of habits they were selected for commencement of treatment. Routine hematological examination was done for all the patients before subjecting them to incisional biopsy for histopathological examination. The biopsies were obtained from the buccal mucosal region in all the cases, since all the cases exhibited clinically evident changes in this area and also taking the consideration of accessibility for biopsy procedures. The specimens were preserved in 10% formalin for further laboratory procedures. The tissue sections were made and studied under microscopy after staining with haematoxylin and eosin. The histopathological grading of OSMF was done according to Pindburg & Sirsat [8].

After histopathological diagnosis of OSMF, 50 clinical stage 2 patients selected for the study. Baseline parameters were recorded. Patients were given 10% curcumin mucoadhesive gel and were instructed to apply topically two times per day making a daily dose of 1 gram.

The primary outcome measures were to note the subjective symptoms like burning sensation, difficulty in mouth opening, intolerance to spicy food and difficulty in swallowing, signs like shrunken uvula, hockey stick appearance of uvula, and objective parameters like blanching, sites of fibrosis, burning sensation, pain, mouth opening, tongue protrusion & cheek flexibility. Patients were explained about visual analog scale (VAS) and were asked to mark the severity of burning sensation (BS) & pain on it. The patients were enquired for the improvement of burning sensation & pain at the subsequent visits and were asked to mark it again on a VAS scale. Burning sensation & pain was then recorded on a percentage reduction basis. The parameters like Interincisal distance (IID), tongue protrusion & cheek flexibility were recorded as mentioned by Ranganathan *et al* [9]. Interincisal distance (IID) was measured with vernier calipers between the right maxillary and mandibular central incisors on maximum opening. If these teeth were missing, they were measured on the corresponding teeth of the left arches. The measurements at subsequent visits were done at the previously recorded sites only, to avoid

misinterpretation. Tongue protrusion was measured with a scale as the distance of movement of the tongue beyond the incisal tips of the lower incisors. Cheek flexibility was measured by a line joining tragus of the ear and angle of the mouth will be drawn. An imaginary perpendicular line from the outer canthus of the ipsilateral eye will be extended downwards to intersect the ala-tragus line using a protractor at 90^{O}. The point of intersection will be marked as a reference point. This will be done on both right and left sides. The distance between the two reference points will be recorded at normal centric occlusion as C_1. The subjects were asked to blow the cheeks fully with lips closed and the distance between the references points will be recorded as C_2. The difference between the 2 values (C_2-C_1) was used as measure of cheek flexibility. The secondary outcome measures were to evaluate the post treatment histopathological changes. All measurements were taken by the same examiner to avoid observer variability.

Follow up

These parameters were analyzed at baseline, 15th day, 30th day, 45th day, 60th day & 75th day, 90th day, 4th month, 5th month and 6th month. Patients were also evaluated histopathologically after 6 months.

Statistical analysis

The data collected were tabulated and analysed. The difference in scores at 15th day, 30th day, 45th day, 60th day, 75th day, 90th day, 4th month, 5th month and 6th month were compared by paired t test. 'p' value of 0.05 or less was utilized for statistical significance.

RESULTS

Age & Sex

14(29.2%) males were in the age range of 11-20 years. There were 22 (45.8%) in the age group of 21-30 years, 11(22.9%) between 31-40 years and 1(2.1%) in 41-50 years. There was 1(50%) female in the age range 0f 31-40 years and 1(50%) female in the age range of 41-50 years. (Table 1)The maximum subjects 22 (45.8%) were in the age group of 21-30 years.

Table 1 Age and Sex wise distribution

Age	Males		Females		Total	
	no	%	no	%	no	%
10-20	14	29.2	0	0	14	28
21-30	22	45.8	0	0	22	44
31-40	11	22.9	1	50	12	24
41-50	1	2.1	1	50	2	4
Total	48	100	2	100	50	100

Habits

Of 50 subjects 7 (14%) chewed areca nut, 40(80%) chewed gutka, 1 (2%) chewed tobacco, 1 (2%) had habit of gutka and smoking and 1(2%) had habit of arecanut and tobacco. (Table 2) It was noted that the majority of subjects in the study chewed only gutka 40(80%).

Table 2 Habit wise distribution

Habits	No. of patients	%
Areca nut	7	14
Gutka	40	80
Tobacco	1	2
Gutka & Smoking	1	2
Areca nut & Tobacco	1	2
Total	50	100

Signs & Symptoms

The study revealed that the maximum number of cases with clinical signs and symptoms were 49 (98 %) patients with difficulty in mouth opening, 48(96%) patients with burning sensation, 33 (66%) patients had intolerance to spicy food, 20(40%) patients had shrunken uvula, 7(14%) had difficulty in swallowing, 2(4%) patients had hockey stick appearance of uvula. (Table 3)

Table 3 Habit wise distribution

Signs & Symptoms	No. of patients	%
Difficulty in mouth opening	49	98
Burning sensation	48	96
Shrunken uvula	20	40
Intolerance to spicy food	33	66
Difficulty in swallowing	07	14
Hockey stick appearance of uvula	02	04

Blanching of Oral Mucosa

50 (100%) patients had blanching in buccal mucosa, followed by 42(84%) in the soft palate, 40(80%) patients had blanching in labial mucosa, 16(32%) patients had hard palate blanching, 9(18%) was noted with tongue, 8(16%) had uvula blanching and 6(12%) had blanching in floor of mouth . (Table 4)

Table 4 Blanching of Oral Mucosa

Blanching of Oral Mucosa	No. of patients	%
Buccal mucosa	50	100
Soft palate	42	84
Labial mucosa	40	80
Hard palate	16	32
Tongue	9	18
Uvula	8	16
Floor of mouth	6	12

Sites of Fibrosis

With regard to the site distribution, buccal mucosa was the most common involved site with 50 (100%) patients demonstrating fibrosis at this site. Retro molar area was the second most common site and affected 48 (96%) of patients, followed by soft palate 30(60%), labial mucosa 26(52%), uvula 22(44%) and tongue 10(20%), 1(2%) floor of mouth. (Table 5)

Table 5 Sites of Fibrosis

Site of Fibrosis	No. of patients	%
Buccal mucosa	50	100
Retro molar area	48	96
Labial mucosa	26	52
Soft palate	30	60
Uvula	22	44
Tongue	10	20
Floor of mouth	1	2

Histopathological Grading

All the 50 clinical stage 2 OSMF patients depending upon the histological features were graded. The grading was done as per the criteria laid down by Pindburg and Sirsat. The following distribution was seen

Very early stage (Grade I): 1 (2%) patients
Early stage (Grade II): 12 (24%) patients
Moderately advanced stage (Grade III): 36(72%) patients
Advanced stage (Grade IV): 1(2%) patients (Table 6)

Table 6 Distribution of patients according to Histopathological grading

Grade	No. of patients	%
Grade I (very early)	1	2
Grade II (Early)	12	24
Grade III (Moderately advanced)	36	72
Grade IV (Moderately advanced)	1	2
Total	50	100

Follow-Up of Patients from Baseline to 6 Months

Signs and Symptoms

The study showed statistically significant reduction (x^2 =230.99, p<0.05) of signs &symptoms from baseline to 6th month. (Table 7)

Sites of Fibrosis

It was noted that there was statistically significant reduction (x^2 =687.58, p<0.05) with the distribution of site of fibrosis when observed from baseline to 6th month. (Table 9)

Mean Scores of Objective Parameters from Baseline to 6th Month

Burning sensation, pain, Mouth opening, Tongue protrusion, Cheek flexibility

For the mean values of burning sensation, pain, mouth opening, tongue protrusion and cheek flexibility scores at baseline, 15th day, 30th day, 45th day, 60thday, 75thday, 90th day, 4th month, 5th month & 6th month refer Table 10.

Comparison of Follow-up results

Comparison Between baseline & 15th day

The study revealed a statistically significant decrease in burning sensation (t=-3.62, p<0.05), statistically non significant reduction in pain (t=-1.18, p>0.05)

Table 7 Signs and symptoms follow-up from baseline to 6th month

Signs and symptoms	Base line		15th day		30th day		45th day		60th day		75th day		90th day		4th month		5th month		6th month	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
BS	48	96	48	96	45	90	38	76	21	42	06	12	02	04	01	02	00	00	00	00
DM	49	98	49	98	49	98	48	96	45	90	42	84	37	74	16	32	04	08	02	04
DS	07	14	05	10	05	10	03	06	00	00	00	00	00	00	00	00	00	00	00	00
ISF	33	66	33	66	19	38	04	08	00	00	00	00	00	00	00	00	00	00	00	00
SU	20	40	18	36	18	36	15	30	13	26	12	24	11	22	12	24	11	22	11	22
HS	02	04	01	02	01	02	01	02	01	02	01	02	01	02	01	02	01	02	01	02
Chi square value, p value and significance	**230.99(p<0.05),Significant**																			

Table 8 Blanching of oral mucosa follow-up from baseline to 6th month

Sites of blanching	Base line		15th day		30th day		45th day		60th day		75th day		90th day		4th month		5th month		6th month	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
BM	50	100	50	100	48	96	48	96	41	82	22	44	15	30	02	04	01	02	01	02
SP	42	84	42	84	42	84	32	64	33	66	29	58	27	54	25	50	24	48	23	46
LM	40	80	37	76	34	68	13	26	04	08	02	04	01	02	-	-	-	-	-	-
HP	16	32	14	28	13	26	06	12	02	04	02	04	-	-	-	-	-	-	-	-
T	09	18	08	16	07	14	07	14	07	14	05	10	03	06	03	06	-	-	-	-
U	08	16	07	14	07	14	07	14	07	14	06	12	05	10	04	08	03	06	03	06
FM	06	12	06	12	06	12	06	12	06	12	04	08	04	08	04	08	04	08	03	06
LM-	-	-	02	04	03	06	22	44	13	26	06	12	-	-	-	-	-	-	-	-
BM-L	-	-	-	-	01	02	01	02	03	06	13	26	14	28	08	16	01	02	01	02
BM-R	-	-	-	-	01	02	01	02	06	12	11	22	13	26	10	20	09	18	05	10
RM	-	-	-	-	-	-	01	02	01	02	-	-	01	02	01	02	01	02	01	02
Chi square value, p value and significance	**479.44(p<0.05),Significant**																			

Table 9 Site of fibrosis follow-up from baseline to 6th month

Sites of fibrosis	Base line		15th day		30th day		45th day		60th day		75th day		90th day		4th month		5th month		6th month	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%
BM	50	100	50	100	49	98	49	96	31	62	14	28	07	14	03	06	01	02	-	-
RM	48	96	48	96	46	92	46	52	40	80	40	80	36	72	27	54	20	40	17	34
SP	30	60	29	58	29	58	25	50	24	48	19	38	14	28	12	24	08	16	10	20
LM	26	52	26	52	24	48	06	12	06	12	-	-	-	-	-	-	-	-	-	-
U	22	44	21	42	21	42	16	32	17	34	15	30	13	26	13	26	12	24	09	18
FM	01	02	01	02	01	02	01	02	01	02	01	02	01	02	01	02	01	02	01	02
T	10	20	10	20	09	18	08	16	08	16	06	12	04	08	03	06	01	02	-	-
HS	-	-	01	02	01	02	01	02	01	02	-	-	-	-	-	-	-	-	-	-
BM-R	-	-	-	-	01	02	01	02	15	30	16	32	17	34	14	28	05	10	03	06
RM-R	-	-	-	-	01	02	01	02	-	-	-	-	03	06	09	18	14	28	14	28
LM-L	-	-	-	-	-	-	17	34	05	10	05	10	-	-	-	-	-	-	-	-
BM-L	-	-	-	-	-	-	-	-	02	04	17	34	15	30	06	12	01	02	01	02
RM-L	-	-	-	-	-	-	-	-	-		-	-	02	04	02	04	03	06	05	10
Chi square value, p value and significance	**687.58(p<0.05),Significant**																			

Blanching of Oral Mucosa

With regard to the blanching of oral mucosa, statistically significant reduction (x^2 =479.44, p<0.05) was observed from baseline to 6th month. (Table 8)

and non-significant increase in mouth opening (t=0.71, p>0.05), tongue protrusion (t= 0.98, p>0.05) and cheek flexibility (t=0.88, p>0.05) when comparison was done between baseline & 15th day. (Table 11)

Table 10 Mean scores of objective parameters from baseline to 6th month

	Burning sensation	Pain	Mouth opening	Tongue protrusion	Cheek flexibility
Baseline	8.7±2.45	5.16±4.73	23.58±2.75	15.4±4.26	7.38±1.55
15th day	7.0±2.23	4.14±3.89	24.0±3.12	16.28±4.69	7.76±2.34
30thday	5.14±2.23	2.93±3.08	24.5±3.34	16.5±4.83	8.00±2.47
45th day	2.84±1.93	1.56±1.88	26.18±3.42	18.00±4.64	9.5±2.55
60th day	1.31±1.52	0.68±1.07	27.48±3.11	19.14±4.35	10.7±2.06
75th day	0.36±0.91	0.03±0.18	29.06±1.35	20.58±4.16	11.86±2.00
90th day	0.08±0.44	00±00	30.74±3.44	21.56±3.70	13.04±1.94
4th month	0.02±0.14	00±00	32.54±3.46	22.76±3.52	14.18±2.09
5thmonth	00±00	00±00	34.48±3.21	24.32±2.94	15.28±2.19
6th month	00±00	00±00	36.52±2.95	25.78±2.57	16.78±2.27

Table 11 Comparison between baseline & 15thday (Paired t-test)

	t-test	P value	Significance
Burning sensation	-3.62	P<0.05	Significant
Pain	-1.18	p>0.05	Not significant
Mouth opening	0.71	p>0.05	Not significant
Tongue protrusion	0.98	p>0.05	Not significant
Cheek flexibility	0.88	p>0.05	Not significant

Comparison Between 15th& 30th day

The study revealed a statistically significant decrease in burning sensation (t=-4.18, p<0.05) and nonsignificant reduction of pain (t=-1.65, p<0.05), and non-significant increase in mouth opening (t=0.77, p>0.05), tongue protrusion (t= 0.23, p>0.05) and cheek flexibility (t= 0.49, p>0.05) from 15th day to 30th day of follow-up. (Table 12)

Table 12 Comparison between 15th & 30thday (Paired t-test)

	t-test	P value	Significance
Burning sensation	-4.18	p<0.05	Significant
Pain	-1.65	P>0.05	Not Significant
Mouth opening	0.77	p>0.05	Not Significant
Tongue protrusion	0.23	p>0.05	Not Significant
Cheek flexibility	0.49	p>0.05	Not Significant

Comparison Between 30th& 45th day

The study revealed a statistically significant decrease in burning sensation (t=-5.52 p<0.05) & pain (t=-2.79, p<0.05.) It was also observed that there was a statistically significant increase in mouth opening (t= 2.49, p<0.05) and cheek flexibility (t= 2.29, p<0.05) and non significant increase in tongue protrusion (t= 1.58 p>0.05) from 30th day to 45th day of follow-up. (Table 13)

Table 13 Comparison between 30th & 45th day (Paired t-test)

	t-test	P value	Significance
Burning sensation	-5.52	p<0.05	Significant
Pain	-2.79	P<0.05	Significant
Mouth opening	2.49	p<0.05	Significant
Tongue protrusion	1.58	p>0.05	Not Significant
Cheek flexibility	2.99	p<0.05	Significant

Comparison Between 45th day & 60th day

The study revealed a statistically significant decrease in burning sensation (t=-4.38, p<0.05) and non significant decrease in pain (t=-1.85, p>0.05)

The study also observed a statistically significant increase in mouth opening (t= 1.98, p<0.05), non significant increase tongue protrusion (t= 1.27, p>0.05) and significant increase in cheek flexibility (t= 2.59, p<0.05) when compared between 45th day & 60th day of follow-up. (Table 14)

Table 14 Comparison between 45th day & 60th day (Paired t-test)

	t-test	P value	Significance
Burning sensation	-4.38	p<0.05	Significant
Pain	-1.85	P>0.05	Not Significant
Mouth opening	1.98	p<0.05	Significant
Tongue protrusion	1.27	p>0.05	Not Significant
Cheek flexibility	2.59	P<0.05	Significant

Comparison Between 60th day & 75th day

The study revealed a statistically significant decrease in burning sensation (t=-3.79, p<0.05) and pain (t=-4.24, p<0.05).The study also observed statistically significant increase in mouth opening (t=2.45, p<0.05) and non significant increase in tongue protrusion (t= 1.69., p>0.05) and cheek flexibility (t= 2.86, p<0.05) when compared between 60th & 75thday of follow-up. (Table 15)

Table 15 Comparison between 60th day & 75th day (Paired t-test)

	t-test	P value	Significance
Burning sensation	-3.79	p<0.05	Significant
Pain	-4.24	p<0.05	Significant
Mouth opening	2.45	p<0.05	Significant
Tongue protrusion	1.69	p>0.05	Not Significant
Cheek flexibility	2.86	p<0.05	Not Significant

Comparison Between 75thday & 90th day

The study revealed a statistically significant decrease in burning sensation (t=-1.98, p<0.05) and non significant decrease in pain (t=-1.34, p>0.05). It was observed that there was a statistically significant increase in mouth opening (t= 2.47, p<0.05), nonsignificant significant increase in tongue protrusion (t= 1.24, p>0.05) and significant increase in cheek flexibility (t= -2.29, p<0.05) when compared 60th& 75thday of follow-up. (Table 16)

Table 16 Comparison between 75th day & 90th day (Paired t-test)

	t-test	P value	Significance
Burning sensation	1.98	p<0.05	Significant
Pain	1.34	p>0.05	Not Significant
Mouth opening	2.47	p<0.05	Significant
Tongue protrusion	1.24	p>0.05	Not significant
Cheek flexibility	2.99	p<0.05	Significant

Comparison Between 90th day & 4thmonth

The study revealed nonsignificant reduction of burning sensation (t=0.29, p>0.05) and no pain with (t=0, p<0.05). It was observed that there was a statistically significant increase in mouth opening (t= 2.61, p<0.05), nonsignificant increase in tongue protrusion (t= 1.68, p>0.05) and significant increase in cheek flexibility (t= 2.82, p<0.05) when compared between 90th & 4thmonth of follow-up. (Table 17)

Table 17 Comparison between 90th day & 4th month (Paired t-test)

	t-test	P value	Significance
Burning sensation	0.92	p>0.05	Not significant
Pain	0.00	p>0.05	Significant
Mouth opening	2.61	p<0.05	Significant
Tongue protrusion	1.68	p>0.05	Not Significant
Cheek flexibility	2.82	p<0.01	Significant

Comparison between 4th & 5thmonth

The study revealed nonsignificant reduction of burning sensation (t=-1.01, p>0.05) and no pain with (t=0, p<0.05). It was observed that there was a statistically significant increase in mouth opening (t= -2.91, p<0.05), tongue protrusion (t= 2.48, p<0.05) and cheek flexibility (t= -2.57, p<0.05) when compared between 90th & 4thmonth of follow-up. (Table 18)

Table 18 Comparison between 4th month & 5th month (paired t-test)

	t-test	P value	Significance
Burning sensation	-1.01	p>0.05	Not significant
Pain	0.00	p>0.05	Significant
Mouth opening	2.91	p<0.05	Significant
Tongue protrusion	2.48	p<0.05	Significant
Cheek flexibility	2.57	P<0.05	Significant

Comparison between 5th & 6thmonth

The study revealed no burning sensation (t=0, p<0.05) and pain (t=0, p<0.05). It was observed that there was a statistically significant increase in mouth opening (t= 3.31, p<0.05), tongue protrusion (t= 2.65, p<0.05) and cheek flexibility (t=3.37, p<0.05) when compared between 5th& 6th month of follow-up. (Table 19)

Table 19 Comparison between 5th month & 6th month (paired t-test)

	t-test	P value	Significance
Burning sensation	0.00	p>0.05	Significant
Pain	0.00	p>0.05	Significant
Mouth opening	3.31	p<0.05	Significant
Tongue protrusion	2.65	p<0.05	Significant
Cheek flexibility	3.37	P<0.05	Significant

Lymphnodes

Comparison Between Baseline & 15th Day, 30th Day, 45th Day, 60th Day, 75thday, 90thday, 4thmonth, 5thmonth and 6th Month

13(26%) patients had bilateral involvement of lymph nodes and 10(20%) of patients had unilateral involvement at baseline. There were no palpable unilateral or bilateral lymph nodes when comparing 60th, 75th, 90thday, 4thmonth, 5th month and 6th month of follow-up results. Overall there was a statistically significant (x^2 = 11.5, p<0.05) change in the involvement of lymph nodes between Pretreatment (Base line) and Post treatment (6th month) follow up. (Table 20)

Table 20 Lymph nodes

Lymph nodes	Unilateral		Bilateral	
	No. of cases	%	No. of cases	%
Baseline	10	20	13	26
15th day	10	20	11	22
3oth day	16	32	02	04
45th day	02	04	-	-
60th day	-	-	-	-
75th day	-	-	-	-
90th day	-	-	-	-
4th month	-	-	-	-
5th month	-	-	-	-
6th month	-	-	-	-
Chi square value, p value and significance	11.5(p<0.05),Significant			

Comparison of Clinical Staging between Pretreatment (Base Line) and Post Treatment (6th Month) Follow Up

There was a statistically significant (x^2 = 100.00, p<0.05) change in the clinical staging between Pretreatment (Base line) and Post treatment (6th month) follow up. (Table 21)

Table 21 Comparison of clinical staging between Pretreatment (Base line) and Post treatment (6th month) follow up

Clinical staging	Pretreatment (base line)		Post treatment (6th month)	
	No. of cases	%	No. of cases	%
Stage I	0	0	50	100
Stage II	50	100	0	0
Total	50	100	50	100
Chi square value, p value and significance	100.00(p<0.05),Significant			

Comparison of Histopathological Grading between Pretreatment (base line) and Post Treatment (6th month) follow up.

There was improvement in histopathological parameters in all the patients which was statistically significant (x^2 = 52.30, p<0.05) when comparison was done between Pretreatment (Base line) and Post treatment (6th month) follow up. (Table 22)

Table 22 Comparison of Histopathological grading between Pretreatment (Base line) and Post treatment (6th month) follow up

Histopathological grading	Pretreatment (base line)		Post treatment (6th month)	
	No. of cases	%	No. of cases	%
Grade I	01	02	10	20
Grade II	12	24	38	76
Grade III	36	72	02	04
Grade IV	01	02	0	0
Total	50	100	50	100
Chi square value, p value and significance	52.30(p<0.05),Significant			

DISCUSSION

The maximum subjects were in the age group of 21-30 years. It was noted that the majority of subjects in the study chewed only gutka. Gutka is a mixture of arecanut, tobacco, lime, catechu and flavouring compounds which are marketed in small sachets or pouches. The habit-forming process of gutka chewers is due to tobacco and areca nut, which if consumed for longer duration and frequencies is responsible for causing addiction, leading to OSMF [10].

Maximum number of cases had blanching in buccal mucosa, followed by soft palate blanching, blanching in labial mucosa, hard palate, tongue, uvula and floor of mouth. Most of the studies showed involvement of different areas in different ratios [11].

Buccal mucosa was the most common involved site. Previous reports also corroborated this findings [12,13,14]. Retro molar area was the second most common site followed by labial mucosa, soft palate, uvula, tongue and lower labial mucosa. Most of the studies showed involvement of these sites also in different ratios [11].

Signs and Symptoms, Blanching of Oral Mucosa, Sites of Fibrosis

The study showed statistically significant reduction of signs & symptoms, blanching and fibrosis when observed from baseline to 6th month. Our study showed similar results with the study of Das *et al* [15] which showed change in color of the oral mucosa from blanched to erythematous. This improvement in blanching could be due to the increase in vascularity brought about by the curcumin regimen [15]. Other authors have not considered blanching in their outcome measure.

Visual analog scale (VAS) was used to record severity of burning sensation (BS) & pain. There was complete reduction of burning sensation & pain which was statistically significant. Statistically significant reduction of burning sensation was also observed in different studies. With inter-group comparison in Kopuri *et al*'s [16] study patients under curcumin group showed a better reduction in severity of burning sensation but did not differ enough to be statistically significant ($P > 0.05$), where as in Das *et al*'s [15] study statistically significant quicker reduction of burning sensation was noted. Agarwal *et al*'s [17] study showed the change in burning sensation on VAS was statistically significant ($P < 0.001$).Hazarey *et al*'s [18] study reported VAS scale with spicy and normal food the average reduction was 64 (42–73) and 77 (70.5–82) as compared to 34 (14.5–64.5) and 64 (46–75.5) respectively in control group.Yadav *et al* [19] reported that burning sensation improved in turmix group at the end of 1st month mean values of 63.5, to 0 at the end of 3rd month. Complete resolution of burning sensation was noted with turmix. Reduction in burning sensation with turmix was statistically significant when compared with conventional therapy ($P < 0.001$).

Pindburg & Sirsat have defined OSMF as juxtraepithelial inflammatory reaction followed by fibro elastic change of lamina propria. So inflammation is definitely a component of OSMF. The amelioration of signs & symptoms could be attributed to the anti-inflammatory property of curcumin [15]. Curcumin offers anti‑inflammatory effect through inhibition of NF-kB activation [20, 21, 22]. Curcumin blocks the IK-mediated phosphorylation and degradation of IBα, thus, NF-kB remains bound to IkBα in the cytoplasm and is not able to enter the nucleus to activate transcription [23]. Curcumin modulates the inflammatory response by down-regulating the activity of cyclooxygenase-2 (COX-2), lipooxygenase, & inhibits the production of the inflammatory cytokines, tumor necrosis factor-alpha (TNF-alpha), interleukin 1, 2, 6, 8, and 12, monocyte chemo attractant protein (MCP), and migration inhibitory protein [19, 21, 24]. Curcumin has been described as a dual inhibitor of arachidonic acid metabolism, as it inhibits both cyclooxygenase & lipooxygenase pathways of inflammation, thus inhibiting the products of inflammation such as prostaglandins & leukotriens [15, 25].

Curcumin inhibits lipid peroxidation using linoleate, a polyunsaturated fatty acid that is able to oxidize and form a free fatty acid radical. Curcumin acts as a chain breaking radical & causes neutralization of lipid radicals. In addition to inhibiting lipid peroxidation, curcumin demonstrates free radical-scavenging activity. It has been shown to scavenge various reactive oxygen species produced by macrophages (including superoxide anions, hydrogen peroxide and nitrite radicals).Inducible nitric oxide synthase (iNOS) is an enzyme found in macrophages that generates large amounts of NO to provide the 'oxidative burst' necessary for defense against pathogens. iNOS is induced in response to an oxidative environment, and the NO generated can react with superoxide radicals to form perioxynitrite, which is highly toxic to cells. It has been shown that curcumin down regulates the iNOS activity in macrophages, thus reducing the amount of reactive oxygen species (ROS) generated in response to oxidative stress [20, 25, 26, 27]

Rai et-al [28] has demonstrated the scavenging effect of curcumin on superoxide radicals, hydroxyl radicals & lipid peroxidation. So the effect brought about by the curcumin could be a synergism of their anti-inflammatory & antioxidant properties. This anti-inflammatory & antioxidant activity of curcumin would have been responsible for statistically significant reduction of burning sensation and pain in our patients. In addition, the antioxidant or free-radical scavenging activity of curcumin also contributes to its anti-inflammatory properties by decreasing the amount of oxidative stress that can trigger the inflammatory cascade [25].

There was statistically significant increase of IID, tongue protrusion & cheek flexibility. There was mean increase of 10.94 mm in IID, 10.38 mm of tongue protrusion, 9.4 mm of cheek flexibility. Similar results were observed in studies done by Rai B *et al* [24], Das AD *et al* [15], Agarwal *et al* [17], Yadav *et al* [19], Hazarey *et al* [18]. Rai B *et al* [28] in their study reported that in patients with submucous fibrosis, mouth opening recovered significantly ($P< 0.05$) after 6 months of treatment. Das *et al* [15] in their study reported statistically significant and equal increase in the mouth opening of patients in Groups I (curcumin capsules) and II (turmeric oil) after 1‑month and 3 months of treatment and also after the follow up period. The mean increase was 0.87 cm in both the groups which was significant when compared with the other groups. Agarwal *et al* [17] in their study mentioned the overall improvement in mouth opening as 0.68mm was not statistically significant ($P=0.109$) this could be because of the shorter duration of treatment for 3 months. In Yadav *et al*'s [19] study the mean increase in IID was 3.13 mm and 1.25 mm respectively in groups 1 & 2. Tongue protrusion (TP) showed greater recovery at the end of 1st month in group 1 when compared with group 2 ($P= 0.004$). Mean increase in TP at the end of the study period was noted to be 2.56 mm and 0.38 mm in group 1 & 2 respectively. Hazarey *et al* [18] in their study reported 5.93 (±2.37) mm increases in mouth opening for test group compared to 2.66 (±1.76) mm of the control group.

Myofibroblasts, typically considered to be activated fibroblasts, play an important role in morphogenesis, oncogenesis, inflammation, wound healing and fibrosis in most organs and tissues. Myofibroblast persistence is a key feature of fibrotic diseases including OSMF, scleroderma, and hepatic, pancreatic, and pulmonary fibrosis. Myofibroblasts can be detected in the OSMF-affected tissues; this phenomenon is related to the severity of OSMF. Myofibroblasts not only synthesize collagen, but also produce numerous inflammatory mediators, chemokines, and growth factors, intensifying and prolonging the inflammation in OSMF by activating the inflammatory corpuscles. This self-excitation of inflammation increases the expression of fibrogenic cytokines such as TGF-β1, and enhances fibrosis. The possibility of inhibiting proliferation and inducing

apoptosis in myofibroblasts offers a new, promising therapy line in the treatment of OSMF [29].

It has been reported in a study that curcumin inhibits cell proliferation in fibroblasts and myofibroblasts. MTT assay revealed that curcumin treatment significantly decreases the proliferation of fibroblasts and myofibroblasts, in a dose-dependent manner. This effect is more pronounced in myofibroblasts; the growth inhibitory rate for myofibroblasts incubated with curcumin was double of that for the similarly treated fibroblasts. Curcumin induces cell cycle arrest in myofibroblasts. Cell cycle analysis shows that curcumin treatment results in a dose-dependent increase in the proportion of myofibroblast cells in G0/G1 phase. Curcumin induces cell apoptosis in myofibroblasts. It has been suggested that mitochondria play a role in curcumin-induced apoptosis [29].

The increase in mouth opening & tongue protrusion could be a result of anti-inflammatory & antioxidant & fibrinolytic properties of curcumin. Curcumin has been reported to possess fibrinolytic action in liver and lung fibrosis in studies conducted by kuttan *et al*. Li *et al* has attributed the fibrinolytic action of curcumin to its three properties namely inhibition of lipid peroxidation, checking cellular proliferation and inhibition of collagen synthesis [15]. This same action of curcumin would also be responsible for the statistically significant reduction of palpable fibrous bands which in turn improves tongue protrusion & cheek flexibility.

There was a statistically significant change in the clinical staging between Baseline to 6^{th} month with p value of <0.05. This was because of overall improvement in all the subjective & objective parameters.

There was improvement in histopathological grading in all the patients which was statistically significant with p value of <0.05 when comparison was done between Pretreatment (Base line) and Post treatment (6^{th} month) follow up. Post treatment (after 6 months) histopathological changes such as hyperplasia of epithelium and reduction in inflammatory cells was observed. These findings correlate with clinical reduction in burning sensation, pain & intolerance to spicy foods. A marked reduction in hyalinization of connective tissue along with reduction in inflammatory cells would have improved the extent of mouth opening, tongue protrusion and cheek flexibility. These findings support the anti-inflammatory and fibrinolytic actions of curcumin. Increase in number of blood vessels (prominent vascular component) presented with significant improvement in color of oral mucosa from blanched to erythematous. There was statistically significant change in the histopathological grading between Baseline to 6^{th} month with p value of <0.05. This was because of overall improvement in all the histopathological parameters. These post treatment histopathological findings were also similar to the study done by Das *et-al* [15]. Other studies have not taken histopathology as their secondary outcome measure.

It is of interest to note that none of the patients presented with malignant transformation. Krishnaswamy reported that curcumin inhibits carcinogenesis by polycyclic aromatic hydrocarbons and hence a prospective chemo preventive agent against oral cancer. Earlier studies have reported that curcumin to be a potent antioxidant and coupled with their ant initiating and detoxifying effects, they have proven to be effective in the chemoprevention of cancer. Along with the inhibition of arachidonic acid metabolism, they also inhibit superoxide generation, thus prevent tumor promotion. Kerry bone has stated that curcumin alters the metabolism carcinogens in liver and increases the activity of detoxifying enzyme glutathione-e-transferase, thus preventing oncogenesis [15]. More recently curcumin has been found to possess anti-cancer activities via its effect on a variety of biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumourigenesis and metastasis. In various studies, anti tumour-promoting effects of curcumin were studied and proved. In these studies it was proved that curcumin showed antitumor-promoting effects due to the induction of apoptosis. Investigations have shown specific inhibitory effect of cyclooxygenase (Cox)-2. In addition, curcumin affects a variety of growth factor receptors and cell adhesion molecules involved in tumour growth, angiogenesis and metastasis [30].

Curcumin also affects both the Phase I and Phase II enzymes of the hepatic cytochrome p450 enzyme system involved in the oxidation and detoxification of toxic substances. Curcumin has been shown to inhibit the Phase I enzymes (including cytochrome p450 isoforms and p450 reductase) which are induced in response to toxin exposure and create a host of carcinogenic metabolites that contribute to DNA adduct formation during the oxidation of such substances. Conversely, curcumin induces the Phase II enzymes involved in detoxification of toxic metabolites (including glutathione S-transferase, glutathione peroxidase and glutathione reductase [25].

All the patients in the study tolerated the treatment regimens well. None of the patients reported any allergic or abnormal reaction nor did elicit any signs and symptoms of toxicity to the treatment modality. The cdri and various studies have reported curcumin to be nontoxic [15].

CONCLUSION

As the number of research studies on the therapeutic effects of Curcumin keeps on increasing across the globe. It could be concluded that Curcumin holds a promising future in local therapeutic applications specific for potentially malignant disorders of oral cavity. This study highlighted that curcumin is safe, non-toxic, effective, with no side effects. Development of novel drug delivery systems such as nanoparticles and solid lipid nanoparticles loaded with curcumin seems to be very promising in enhancing its efficacy in addition to its stability. Moreover, future research is required to determine the long-term effects of curcumin on a large number of subjects clinically.

Funding resources

There is no funding resource for the study.

Conflict of interest

There are no conflicts of interest for the study.

Ethical approval

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institution. Informed consent was obtained from all individual participants included in the study.

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