



Research Article

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Effect of Turmeric on Serum Malondialdehyde levels in Oral Submucous Fibrosis

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Abstract

Background: Aspergillus flavus in betel nut secretes aflatoxin i.e. mycotoxin & tobacco which leads to production of free radicals and reactive oxygen species, thus increasing lipid peroxidation. Biological parameter of Lipid peroxidation is malondialdehyde. Hence present study was conducted to evaluate effect of turmeric on increased lipid peroxidation by using serum malondialdehyde levels & as a surrogate outcome on clinical signs & symptoms of oral submucous fibrosis (OSMF). **Methods:** In this study 270 subjects of which 30 were suffering from OSMF on turmeric with counseling them about stoppage of habit (group A), 30 subjects suffering from OSMF on turmeric without counseling them about stoppage of habit (group B), 30 subjects suffering from OSMF counseled for stoppage of habit but without turmeric medication (group C), 90 cases which were betel-nut habitual without OSMF (group D), 90 cases healthy control group (group E). **Results:** Statistically significant reduction in Serum MDA levels post treatment group A patients as compared to group B & group C ($p < 0.05$). Mean difference was observed as 9.00 in group A, 3.81 in group B, 2.62 in group C. **Conclusion:** Increased serum MDA in OSMF can be used as an important marker in early diagnosis, treatment, prognosis and for prevention and clinical intervention of the disease. The antioxidant properties of turmeric reduce the oxidative stress even with effects of areca alkaloids. Serum MDA level was decreased in subjects taking turmeric medication for the period of 4 weeks.

Keywords: OSMF, betel-nut, lipid peroxidation, Malondialdehyde.

INTRODUCTION

Oral Submucous Fibrosis (OSMF) is defined as an "Insidious chronic disease of oral mucosa characterized by inflammation and progressive fibrosis of lamina propria and deeper connective tissues, followed by stiffening of an otherwise yielding mucosa resulting in difficulty in opening mouth & inability to eat." Overall prevalence of OSMF in India is about 0.5% [1,2].

Betel nut habit is principle culprit in pathogenesis & development of OSMF. Ingestion of chillies, nutritional deficiency, autoimmunity and genetic susceptibility, immunologic process and nutritional deficiencies are considered as cofactors [3].

Interaction of free radicals & reactive oxygen species (ROS) with lipid, DNA & proteins is responsible for degradation of proteins & promote DNA strand breakage & harms genomic structure. Free radicals are continuously produced as a result of chain reaction in lipid peroxidation leading to formation of malondialdehyde (MDA), propanedial, 4 hydroxynonenal (4-HNE) [4]. MDA had been proved as an important biological parameter for tissue injury caused by free radical-mediated lipid peroxidation [5].

Antioxidants are biological substances that safely remove ROS which are continuously formed during normal aerobic metabolism [6].

Turmeric is a polyphenol derivative of Curcuma longa plant. Curcumin (diferuloylmethane) chief component of turmeric is a carotenoid pigment which has been used extensively in ayurvedic medicine for centuries, as it is nontoxic and has a anti-oxidant, analgesic, anti-inflammatory, antiproliferative, anti-angiogenesis, apoptosis inducing & antiseptic activities [7-10].

Aim of the present study was to determine effect of turmeric on lipid peroxidation in OSMF thus evaluating antiprecancerous effects of turmeric.

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MATERIALS AND METHODS

This study was conducted after obtaining ethical approval from institutional ethics committee, DMIMS (DU) in our department with contribution of Mahatma Gandhi Ayurvedic College & Hospital, Salod, Wardha and Central Research Lab, Jawaharlal Nehru Medical College, Sawangi (M), a sister institute of DMIMS (DU) in our study. Detailed case history of patients was taken & thorough examination was done. Clinical findings were recorded in a structured case history proforma after taking their consent.

All patients were included from OPD of Oral Medicine & Radiology. In group A, 30 OSMF subjects were included those visited on every Monday, in group B on every Wednesday, of group C on every Friday. Habitual controls & healthy controls were selected from persons accompanying with OSMF cases. Inclusion Criteria was patient's above age of 18 clinically newly diagnosed as having OSMF [11]. Two samples of raw turmeric were purchased & estimated for concentration of curcumin by HPLC. Preparation of turmeric powder having more concentration of curcumin done using pulverization procedure. 500 mg turmeric capsules were dispensed in plastic bottles containing 60 each. Calculated sample was having 59 mg of curcumin in 1000 mg of turmeric. Thus 59 mg of curcumin per day was given to patient. Patients in group A & group C were counseled for strict complete stoppage of habit but later not on turmeric. In group B, subjects on turmeric with continuation of habit. Patients were evaluated at end of 4th week of treatment. Venous blood samples were collected pretreatment & posttreatment for MDA estimation.

VAS scale was used encoding 10 scores according to severity of symptoms regarding patient's perception of Burning Sensation to spicy foods, Taughtness of fibrous bands, Roughness of mucosa, Ulceration, Tongue movement pretreatment & posttreatment.

Serum MDA was assayed [12]. MDA concentration was calculated as $\eta\text{mol/ml}$ pre-treatment & post-treatment serum MDA level was estimated in groups A, B, C & in group D, E only single sample was estimated for MDA (habitual control) & E (healthy control).

Statistical Analysis

Statistical Analysis was done by using descriptive and inferential statistics using one-way ANOVA, Tukey Multiple Comparison Test and Student's paired t test.

RESULTS

The mean MDA level in the group A, group B, group C, group D & group E was found to be $20.79 \pm 7.15 \eta\text{mol/ml}$, $21.0470 \pm 5.84 \eta\text{mol/ml}$, $22.72 \pm 6.33 \eta\text{mol/ml}$, $9.3650 \pm 1.055 \eta\text{mol/ml}$, $5.80 \pm 1.77 \eta\text{mol/ml}$ respectively. Thus lipid peroxidation levels were increased in OSMF patients as compared to habitual & healthy controls (Chart 1).

Pre-treatment mean MDA level in group A was estimated to be $20.79 \pm 7.15 \eta\text{mol/ml}$ & at post-treatment it was found to be $11.79 \pm 4.76 \eta\text{mol/ml}$. Mean MDA level in group B was found to be $21.04 \pm 5.84 \eta\text{mol/ml}$ pre-treatment & at post-treatment it was found to be $17.23 \pm 5.35 \eta\text{mol/ml}$. Mean MDA level in group C was found to be $22.72 \pm 6.33 \eta\text{mol/ml}$ pre-treatment & at post-treatment it was found to be $20.09 \pm 6.07 \eta\text{mol/ml}$. Thus significant decrease in Serum Malondialdehyde levels (lipid peroxidation) post-treatment were found in group A patients (mean difference -9.00) as compared to groups B (mean difference - 3.81) & C (mean difference - 2.62). (Chart -2)

Significant increase in interincisal mouth opening, reduction in burning sensation, reduction in taughtness of fibrous bands, reduction in ulcerations observed in group A pts as compared to group B after giving whole turmeric capsules as a part of medication along with stoppage of habit ($p < 0.05$). [Charts - 3, 4, 5, 6 respectively]

DISCUSSION

Oral Submucous Fibrosis is a chronic inflammatory irreversible oral mucosal condition. OSMF is mainly caused by arecoline which is areca nut alkaloid. Arecoline induces increased proliferation of fibroblasts leading to increased collagen formation which is responsible for the development of OSMF [13]. Aflatoxins in contaminated betel nuts cause oxidative stress by increasing lipid peroxidation and decreasing enzymatic and non-enzymatic antioxidants in aflatoxin-treated animals [14].

The free radical especially Hydroxyl radical is an extremely aggressive oxidants that can damage most of biological molecule of cell by lipid peroxidation, oxidative modification of proteins, DNA base alteration. MDA is frequently used biomarker that provides overall information regarding lipid peroxidation [15]. Evaluation of serum MDA level may be regarded as potential surrogate marker for evaluating disease process of Oral submucous fibrosis.

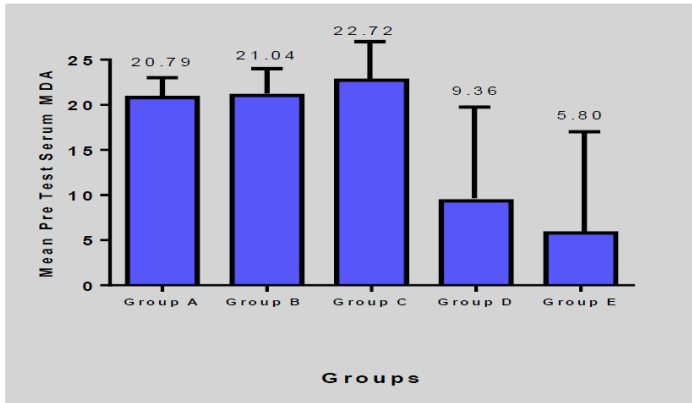
Studies have reported that antioxidants like vitamin A, C, E, β carotene causes toxic reactions in body on excess consumption. Whole turmeric has no such adverse effects on body upto 10 g/day. Tolerance level of curcumin is 12 g per day which was previously proven in phase I three different human clinical trials [16].

Plasma MDA levels in OSMF ($3.3 \pm 0.4 \eta\text{mol/ml}$) were significantly found to be increased when compared to that in healthy individuals (2.4 ± 0.5) ($P < 0.001$). Significant improvements and amelioration of symptoms were observed on follow up of OSMF patients after 6 wks of treatment with "antoxid" tablets 1 tablet thrice daily. Significantly decreased mean MDA levels and increased levels of beta-carotene were observed ($P < 0.001$) [17].

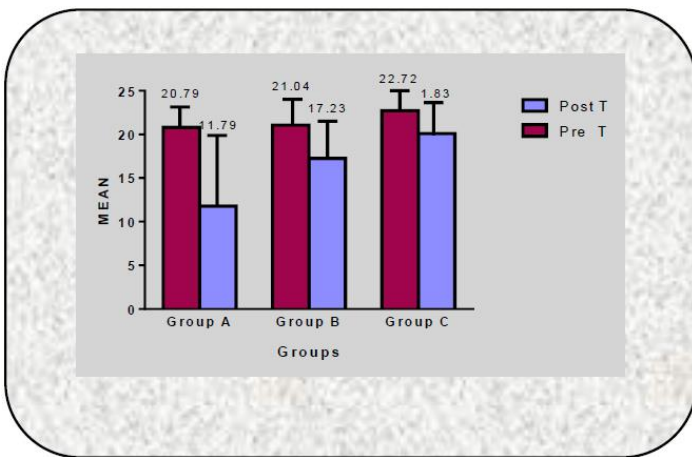
On interrelating serum MDA levels, Superoxide dismutase (SOD), Vitamin A similar results were observed previously in a clinical & histopathological study of OSMF [18]. Mean serum MDA level in healthy individuals was $5.107 \pm 2.32 \eta\text{mol/ml}$, in oral precancer $9.33 \pm 4.89 \eta\text{mol/ml}$ and in oral cancer $14.34 \pm 1.43 \eta\text{mol/ml}$ found as a result in a histopathological study [19].

Similarly we observed that, pretreatment serum MDA level was increased in OSMF patients when compared to normal healthy individuals, [Graph - 1]. This shows possibility of role of extent of lipid peroxidation i.e. oxidative stress & ROS in aetiopathogenesis of oral precancer. In present study we observed that there is significant reduction in serum MDA levels i.e. lipid peroxidation posttreatment in all three groups (A, B, C) ($p < 0.05$) with more reduction is observed in group A as compared to group B [Graph- 2]. This showed that stoppage of habit helps in reducing lipid peroxidation but stoppage of habit with turmeric medication had significant effect on lipid peroxidation (MDA) & clinical signs & symptoms. In group B patients, we observed turmeric medication showed its antioxidant & anti-inflammatory properties irrespective with habit. On comparing habitual controls & healthy controls (groups D & E), increased MDA level in habitual controls indicates that lipid peroxidation is initiated by betel nut products with or without tobacco well before clinically apparent disease process of OSMF.

Dissolution of Fibrin as a characteristic action of curcumin had been studied previously in liver and lung fibrosis [20].



Graph 1: Comparison of Serum Malondialdehyde levels (Mean) in all groups



Graph 2: Comparison of Serum Malondialdehyde levels (Mean) at pre and post treatment in three groups (A, B, C)

Iron chelate complex was formed which was later excreted by including chemo-protective agents like turmeric, clove, chili, or cardamom (2%) in the diet which resulted in improvement in liver functions [21].

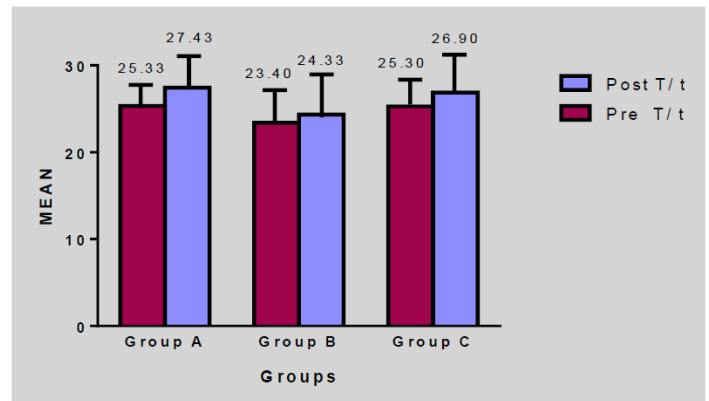
Dietary curcumin and capsaicin were combinely found to inhibit in vivo iron-induced LDL oxidation and in vitro copper-induced LDL oxidation [22]. Curcumin and capsaicin combinely protect LDL oxidation process significantly more than independently.

Curcumin showed significant reduction in clinical signs & symptoms in patients with oral Leukoplakia [23]. Reduction of serum MDA levels was observed between pretreatment & posttreatment at end of 4 weeks of treatment with 500 mg turmeric capsules. Significant results were observed in group A as compared to B & C. [Graph – 2].

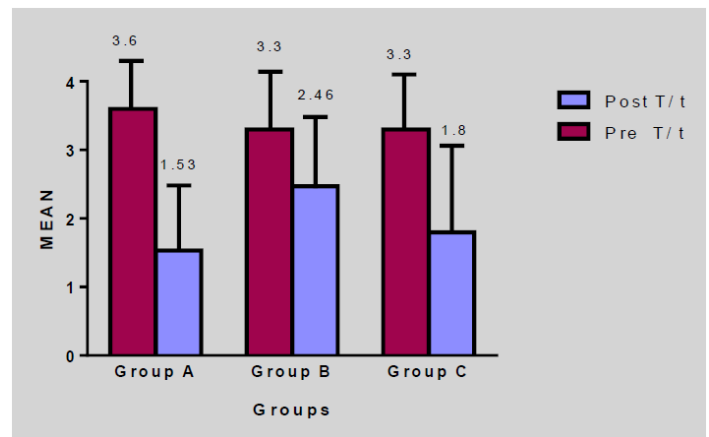
Curcumin had shown to suppress a response to chewing, sniffing form of tobacco contents or nitrosamine ketone cyclooxygenase-2 (nicotine derivative) in oral premalignant and cancer cells in vitro [24].

In an animal study of malignancies caused by chemical carcinogens, turmeric extract and turmeric oil proved to be chemoprotective in the doses of 0.6 ml and 1.0 ml resp. for 3 months. In the similar manner, human clinical trials can be assessed for evaluation of chemoprevention activity of both these agents in OSMF [25]. Curcumin capsules and turmeric oil had shown appreciable improvement in the clinical outcomes of OSMF within one month of treatment, thus it appears to be a promising approach in the management of OSMF patients [26]. Topical application of turmeric extract as an ointment twice/day for 3 months resulted in beneficial clinical outcomes in patients with oral lichen planus [27]. Significant clinical improvements & better functional outcomes were recorded in OSMF patients, as a progressive jaw opening after treatment with turmeric and turmeric oil [28]. Similarly statistically significant increase in interincisal opening & reduction in burning

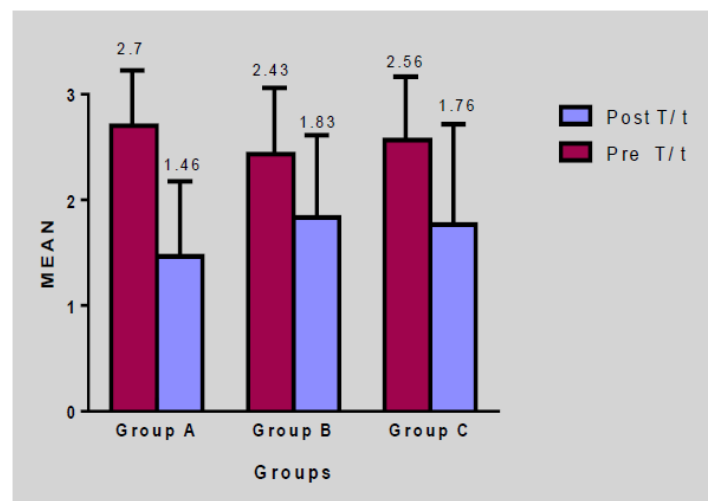
sensation was observed in groups A, B & C but more significant in group A patients who were on turmeric medication & with complete stoppage of habit. [Graph- 3,4].



Graph 3: Comparison of Interincisal opening levels (Mean) at pre and post treatment in three groups



Graph 4: Comparison of Burning sensation to spicy foods (Mean) at pre and post treatment in three groups (A,B,C)

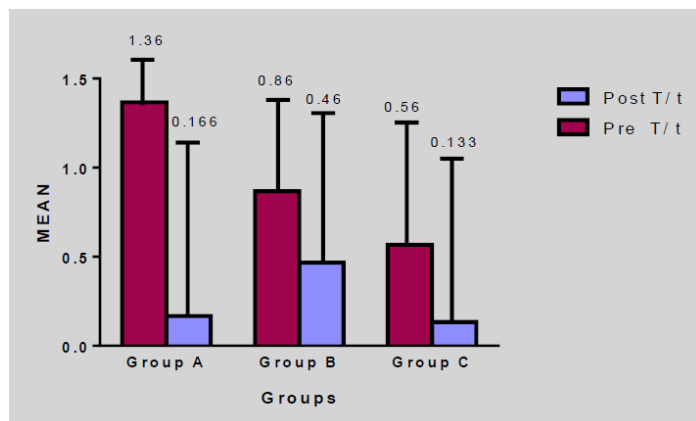


Graph 5: Comparison of Taughtness of fibrous bands (Mean) at pre and post treatment in three groups

Curcumin had shown dose dependent complete blockage of arecoline induced fibrinogenesis in a vivo study on human buccal mucosal fibroblasts in OSMF. Thus their study results suggest that curcumin could be a useful agent in controlling OSMF in vivo [29]. This suggests its fibrinolytic activity. Similarly in present study, significant reduction in taughtness of fibrous bands i.e. clinical improvement in consistency of

fibrous bands was observed in group A patients as compared to groups B & C & was directly proportional to level of serum MDA. [Graph- 5].

In peptic ulcer patients, 2 capsules of turmeric (300 mg each) 5 times daily for 4 weeks showed improvement in a healing rate upto 48% [30]. Similarly in present study, we observed reduction in ulcerations of oral mucosa in 40% of OSMF patients (groups A & B). Significant reduction is observed in group A after giving turmeric capsules & with stoppage of habit. ($p < 0.05$) [Graph- 6].



Graph 6: Comparison of ulcerations (Mean) at pre and post treatment in three groups

Thus, turmeric not only inhibits the progression of carcinogenesis process but also causes reversal of the precancerous conditions like OSMF.

CONCLUSION

Serum MDA level has increased in oral submucous fibrosis patients as compared to normal individuals, which has observed to be decreased on taking turmeric medication for the period of 4 weeks. Thus level of lipid peroxidation i.e. oxidative stress gets decreased due to turmeric supplementation. Curcumin has outstanding antioxidant, anti-tumor, anti-inflammatory, antiviral, antibacterial, antifungal, analgesic, anti-allergic, antioxidant and antiseptic properties which signify its potential use in treatment of various diseases including oral Potentially Malignant Disorders (PMDs) like OSMF. Thus, this choice of therapy accompanied with stoppage of habit is beneficial, affordable and noninvasive to patients with OSMF to control the malignant transformation.

Conflict of Interest

The author reports no conflicts of interest.

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