Original Article

Randomized controlled trial to evaluate the efficacy of oral lycopene in combination with vitamin E and selenium in the treatment of oral leukoplakia

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ABSTRACT

Aims and Objectives: A randomized controlled study was carried out to evaluate the efficacy of lycopene in combination with vitamin E and selenium in the treatment of oral leukoplakia in patients visiting the Government Dental College and Hospital, Mumbai. **Materials and Methods:** Forty-one patients of leukoplakia were randomly categorized (irrespective of size and severity of the lesions) in two groups: Group A and B. Group A consisting of 21 patients were administered combination of lycopene (3 mg), vitamin E (200 I.U.) and selenium (100 mcg) twice daily and group B consisting of 20 patients were given placebo capsules once daily for a period of 3 months. Post-treatment follow-up period was 3-4 months. The product used in the study was LYC-O-MATO soft gels, manufactured by Mano pharmaceuticals, Chennai, India. The treatment outcome was evaluated both clinically and histologically and the results were statistically evaluated using Student's unpaired '*t*' test. **Results:** The results showed that the patients receiving lycopene in combination with vitamin E and selenium have statistically significant improvements both clinically and histologically as compared to those receiving placebo and with no side effects. **Conclusion:** The study results proved the efficacy and safety of lycopene along with selenium and vitamin E in the management of oral leukoplakia.

Key words: Lycopene, oral leukoplakia, selenium, vitamin E

Introduction

Oral cancer is a progressive disease known to involve a series of recognizable stages called 'the multistep process of tumorogenesis'. Tobacco use, in any form, is implicated as an important risk factor in the development of oral cancer. In the long incubation period between the initiation of carcinogenic tobacco habits and the development of invasive oral cancer, well-defined precancerous or premalignant lesions/conditions occur.[1] Leukoplakia is by far the most common oral premalignant lesion representing 85% of such lesions. Oral leukoplakia is associated with tobacco habit and cannot be characterized as any other lesion.[2] The main purpose of early identification of leukoplakia is early interception and management to prevent its malignant transformation. Early neoplastic or preneoplastic lesions can be treated through "cancer chemoprevention" before development of clinically apparent signs and symptoms in overt malignancies wherein the carcinogenic steps can be arrested or reversed through pharmacological treatments.[3]

Various antioxidants like retinol, retinoid, carotenoids, ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E)

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had been used as effective chemopreventive agents in the treatment of leukoplakia.[4] Lycopene, a carotenoid that gives tomatoes, and other fruits and vegetables their red color, is a potent antioxidant with a singlet oxygen quenching ability twice which is that of beta-carotene and 10 times higher than alpha-tocopherol.[5] It enhances the gap junctional communication (GJC) between the cancer cells and increases the resistance of lymphocytes to oxidative stress.[6] The inverse relationship between high tomato consumption and upper aero-digestive tract cancers like cancers of oral cavity, pharynx, larynx and esophagus has been proved in several studies.[7,8] One such study suggested that lycopene can be effectively and safely used for the management of oral leukoplakia.[9] Also there are many previous studies, showing the efficacy of vitamin E and selenium, in the treatment of oral leukoplakia.[10,11] The following study evaluates the efficacy of lycopene in combination with vitamin E and selenium in the management of oral leukoplakia clinically and histologically.

Materials and Methods

A total of 60 patients, who visited the Department of Oral Medicine and Radiology at the Government Dental College and Hospital, were selected for the study, where oral leukoplakia was confirmed both clinically and histologically. Of these, 41 patients successfully completed the trial and reported for follow-up. An ethical clearance was obtained from the ethical committee of the hospital. The aim and purpose of the study was explained to each patient thoroughly and written consent was obtained. Also the habits like tobacco chewing and ethanol usage were assessed on each visit and the patients were encouraged to discontinue the same.

These 41 patients were randomly categorized (irrespective of the size and severity of the lesions) in two groups:

- Group-A (Study group): Twenty-one patients were treated with 6 mg of lycopene + vitamin E (400 I.U.) + selenium (200 mcg) in two equally divided doses. The product used in the study was LYC-O-MATO soft gels, manufactured by Mano pharmaceuticals, Chennai, India.
- Group-B (Placebo group): Twenty patients were given placebo capsules once daily.

After recording the pre-treatment clinical and histological findings, all the patients were evaluated at a regular interval of 15 days for a period of 3 months of active treatment and once in a month for another 3 months of post-treatment follow-up. In case of any untoward reaction such as rash, allergy, etc., the patients were asked to report immediately. During each visit, the clinical response was evaluated by bi-dimensional measurement of the lesions and color photography. The clinical response was noted down carefully and was classified as following:

- Complete remission of lesion (100%).
- Partial improvement or decrease in the size of the lesion more than 50% (75%).
- A stable or no response when size reduction is less than 50% (no response).
- Progression (Prog) or appearance of a new lesion (–25%).

Post-treatment biopsy was taken after the completion of 3 months of treatment period. The histological examination of pre- and post-treatment sample was done in a blind fashion by the same examiner. The histological response was categorized according to the degree of dysplasia as:

1. Atypical hyperplasia.
2. Mild dysplasia.
3. Moderate dysplasia.
4. Severe dysplasia or carcinoma *in situ*.

And they were ranked according to severity as follows: Normal as 0, atypical hyperplasia as 1, mild dysplasia as 2, moderate dysplasia as 3 and severe dysplasia or carcinoma *in situ* as 4. All responses were calculated in units, e.g., if moderate dysplasia became mild dysplasia after treatment, then an improvement of 1 unit was considered (3 minus 2). Statistical analysis was done and results were compared using Student's (unpaired) 't' test.

Results

In this study, the majority i.e., 38 (92.6%) were males. There were 19 males and two females in group A and 19 males and one female in group B. Thus the male predominance over females was as high as 12:1 [Tables 1 and 2]. Of the 41 patients in the two groups (group A + group B) the average age was found to be 47 years. Most of the patients were in the middle age (31-50 years). The youngest patient in this study was 26 years old and the oldest was 65 years old.

The site involved in the majority of patients was buccal mucosa. Thirty-six (88%) patients out of 41 in general (group A + B) had lesions on buccal mucosa followed

Table 1: Age and sex distribution in group A

Age group (in years)	Male	Female	Total	Age in %
11-20	—	—	0	0
21-30	1	—	1	4.76
31-40	5	1	6	28.57
41-50	7	—	7	33.33
51-60	4	—	4	19.05
61-70	2	1	3	14.29
Total	19 (90.5%)	2 (9.5%)	21	—

by the gingiva/ridge (12.19%), tongue (9.75%), lips (7.31%) and palate (2.43%) [Table 3]. The most common clinical type of leukoplakia observed in patients was homogenous followed by non-homogenous and verrucous type [Graph 1].

The mean average size of the lesion (in cm^2) before treatment between the two groups was comparable [Table 4]. In the study group the average size of leukoplakia before treatment was 9.85 cm^2 (S.D. = 5.87) and after treatment, it was 1.49 cm^2 (S.D. = 3.08). In the placebo group, it was 7.58 cm^2 (S.D. = 7.5) before treatment and after treatment it was 8.55 cm^2 (S.D. = 7.69). Post-treatment it was found that there was a drastic reduction in the size of the lesion in the study group but not in the control group.

When Student's '*t*' test was used to compare pre- and post-treatment results in group A, the improvement was highly significant while the post-treatment improvement in group B was not very significant. Improvement in size was recorded fortnightly which also clearly showed significant improvement in the study group compared to the placebo group [Graph 2].

On statistical evaluation, the patients in group A (lycopene + vitamin E + selenium) showed a mean improvement of 85% with a standard deviation of 24.77 and in group B, it was 16.5% with a standard deviation of 24.78. Five patients showed complete (100%) improvement (CI), 14 showed partial (>50%) improvement (PI) and two showed stable response (<50%) or no improvement (SR) in group A while in group B only three patients showed partial improvement, 17 showed no improvement or stable response, and not a single patient showed complete improvement. The response was highly significant when group A was compared with group B [Table 5 and Graph 3].

Table 2: Age and sex distribution in group B

Age group (in years)	Male	Female	Total	Age in %
11-20	—	—	0	0
21-30	1	—	1	5
31-40	7	—	7	35
41-50	6	1	7	35
51-60	3	—	3	15
61-70	2	—	2	10
Total	19 (95%)	1 (5%)	20	—

Table 3: Site distribution of the lesions

Site	Group A	Group B	Total (%)
Upper lip	—	—	0 (0)
Lower lip	3	—	3 (7.31)
Left buccal mucosa	9	11	20 (48.78)
Right buccal mucosa	8	8	16 (39)
Gingiva and Ridge	2	3	5 (12.19)
Palate	1	—	1 (2.43)
Tongue	2	2	4 (9.75)
Floor of mouth	—	—	0 (0)

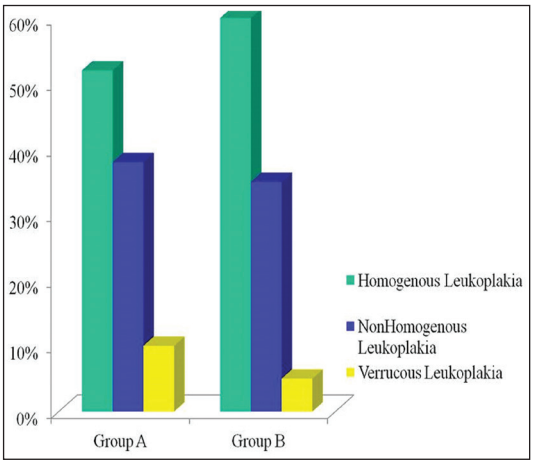
Table 4: Clinical improvement in size (cm^2) of the lesion

Groups	Values	Pre-treatment (in cm^2)	Post-treatment (in cm^2)	Improvement (in cm^2)	*t*-value
Group A	Mean	9.85	1.49	8.36	6.59
	S.D.	5.87	3.08	5.81	
Group B	Mean	8.55	7.58	0.98	3.49
	S.D.	7.69	7.50	1.25	

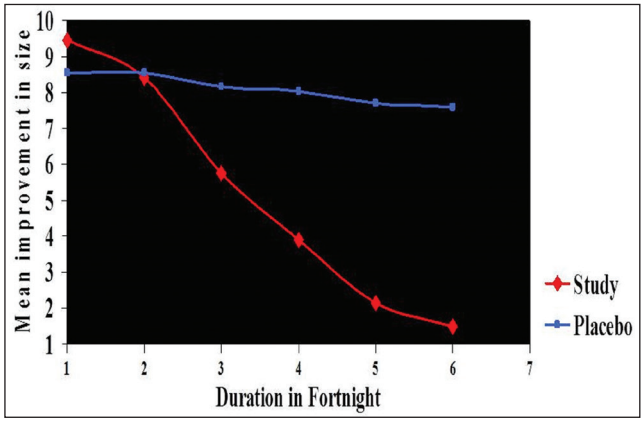
Table 5: Clinical improvement in percentage (%)

Improvement	Number of patients (Group A)	Number of patients (Group B)
Complete improvement (100%)	5	0
Partial improvement (>50%)	14	3
Stable response (<50%)	2	17
Progression (-25%)	0	0
Mean	85%	16.5%
S.D.	24.77	24.78

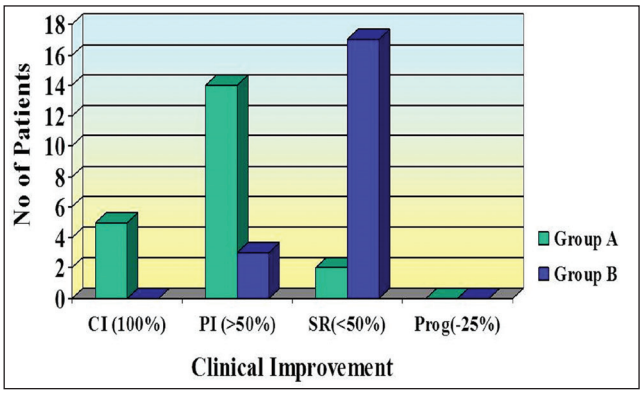
t-value = 8.86, *P* = 0.00052



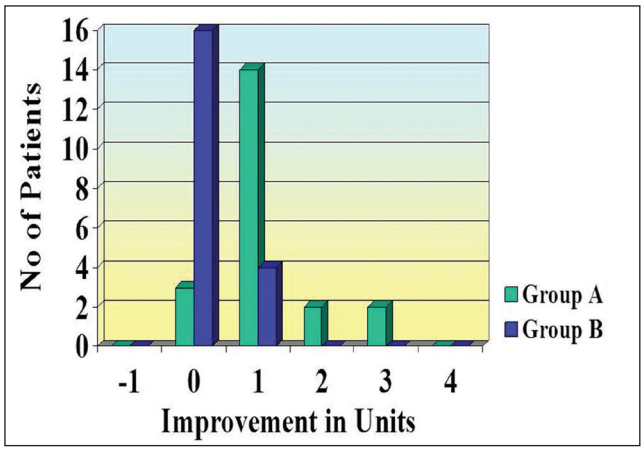
Graph 1: Types of leukoplakia in both groups



Graph 2: Clinical improvement in size measured fortnightly



Graph 3: Clinical improvement in percentage (%)



Graph 4: Histological improvement

Table 6 and Graph 4 show the histological improvement in both the groups. It mentions the actual number of patients with histological stages such as atypical hyperplasia, mild dysplasia, moderate dysplasia and severe dysplasia, and their improvement after treatment. On histopathological evaluation, it was observed that 14 patients in the study group and only four patients in the placebo group showed 1 unit improvement while no improvement was found in three patients of group A (study group) and 16 patients of group B (placebo group) [Table 7]. Histological response or improvement in the study group, including both partial and complete was 86% (18 out of 21) as compared to 20% (4 out of 20) in the placebo group. Student's '*t*' test was used to compare the histological improvement in study group and the placebo group. This test proved that histological improvement in group A was significant when compared to group B.

Table 6: Histological improvement

Groups	Histological stage	Number of patients pre-treatment	Number of patients post-treatment
Group A	Atypical hyperplasia	6	N-5 AH-1
	Mild dysplasia	10	N-1 AH-6 MD-2
	Moderate dysplasia	5	N-2 AH-1 MD-2 MOD-O
	Severe dysplasia	—	—
Group B	Atypical hyperplasia	9	N-0 AH-9
	Mild dysplasia	9	N-0 AH-4 MD-5
	Moderate dysplasia	2	AH-0 MD-1 MOD-1
	Severe dysplasia	—	—

N = Normal, AH = Atypical hyperplasia, MD = Mild dysplasia, MOD = Moderate dysplasia

Table 7: Histological response of group A and B (in units)

Unit of improvement	Group A	Group B
−1	—	—
0	3	16
1	14	4
2	2	0
3	2	0
4	—	0
Mean	1.14	0.2
S.D.	0.793	0.41
Percentage	86%	20%

t-value = 4.75, *P* = 0.0057

Discussion

Leukoplakia was defined by the World Health Organization (WHO) in 1978 as a white patch or plaque which cannot otherwise be characterized clinically or pathologically as any other disease. Leukoplakia is more prevalent among older and elderly men and its prevalence increases with age.[12] In our study 95% of patients were above 30 years, while the mean age was 47 years. Also, the male to female ratio was 12:1 indicating a high male predominance. Various other investigators like Waldron,[13] Banoczy[14] and more recently Mohitpal Singh *et al*.[9] also reported male predominance.

In the present study it was found that lycopene in combination with vitamin E and selenium was effective in improving both clinical and histopathological parameters in the patients of leukoplakia and the response was statistically significant as compared to the placebo group. The first clinical response noted was thinning of the leukoplakic lesion or keratosis, followed by decrease in the size of the lesion and the appearance of pink mucosa. Histological response in patients receiving lycopene was significant and was marked by a reversal of various dysplastic changes.

Mohitpal Singh *et al*., in 2004,[9] assessed the efficiency of lycopene in 58 cases of leukoplakia. In their study, the patients were divided into three groups, and received 8 mg/day, 4 mg/day, and a placebo for a period of 3 months. The study suggested that lycopene could be effectively and safely used for the management of oral leukoplakia. Also, the 8-mg regime showed better results than the 4-mg regime. The efficacy of lycopene as a potent antioxidant in the treatment of other oral precancerous lesions like oral submucous fibrosis has also been proved in many studies.[15,16] Many *in vivo* studies carried out in animals by investigators like Nagasawa *et al*. (1995),[17] Sharoni (1997),[18] Narisawa *et al*. (1998)[19] and Bhuvaneshwari (2001)[20] have shown lycopene to be effective in inhibiting carcinogenesis.

Other similar chemopreventive drugs like retinoids and beta-carotene also have been successfully used in the treatment of leukoplakia but the biggest drawback was toxic reactions like cheilitis, dryness, peeling of skin or headaches related to their provitamin A activity. Lycopene lacks the beta-ionone ring structure and is therefore devoid of pro-vitamin A activity and related side effects.[21] In our study, during the period of active treatment of 3 months, no patient reported with undesirable side effects proving the safety of drug in the management of leukoplakia.

Conclusion

The rising incidence and mortality rates have made prevention of head and neck cancer a research priority. One great arm of this research is cessation of tobacco habits, since tobacco is the greatest single cause of this disease. Another major arm of this research is chemoprevention. Numerous clinical strides have been made in understanding the role of various chemopreventive agents, particularly carotinoids and micronutrients in the treatment of oral cancer. Although the present study clearly proves that lycopene in combination with vitamin E and selenium is an effective and safe chemopreventive agent, the fact that it is based on a small sample size and short period of follow-up, cannot be neglected. The chemopreventive agents must possess minimal toxicity for long-term therapy. Further studies can also be carried out using genetic markers like EGFR, P-53, to assess the efficacy of chemopreventive agents more accurately. Hence, it is recommended that a similar study utilizing a larger sample size, genetic markers, longer follow-up and multiple observer examination of the lesions should be carried out to establish the chemopreventive action of lycopene beyond doubts.

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