The Role of Free Radicals in the Various Oxidation Processes in the Body

Lycopene is a safe antioxidant of utmost importance. Lycopene is a bright red carotene and carotenoid pigment and phytochemical found in tomatoes and other red fruits and vegetables, such as red carrots, watermelons and papayas.

**Objectives:** The main aim and objective of this study is to evaluate the clinical and histopathological response of oral submucous fibrosis to Lycopene—a carotenoid antioxidant.

**Methods and Materials:** The material for this study constitutes 12 adult patients picked from the regular outpatients attending the Department of Dentistry, Hassan institute of Medical Sciences, Hassan, Karnataka, India. Clinically, they are diagnosed as having oral submucous fibrosis.

**Conclusions:** Responses are assessed clinically and histopathologically. We observed clinical and histological improvement in various parameters used in our study. No significant toxicity attributable to Lycopene is encountered in this study.

**Key Words:** Lycopene, Antioxidant, Submucous Fibrosis, Free Radical theory

**INTRODUCTION**

Oral submucous fibrosis (OSMF) has a very interesting history. Sushrutha, a renowned Indian physician who lived in the era from 2500 to 3000BC, had already recognized it as a mouth and throat malady and had labeled it as Vidhari [1]. The features of which were described as a progressive narrowing of the mouth, blanching of the oral mucosa, pain and burning sensation on taking food, hypomobility of the soft palate and tongue, loss of gustatory sensation and occasional mild hearing impairment due to the blockage of the Eustachian tube [2, 3]. There has been nearly no change in these symptoms till today. This condition has a reference in modern literature due to the works of Schwartz in 1952. Joshi was the first to describe this condition in India in 1952 and he coined the name 'oral submucous fibrosis’ [4]. The aetiological studies on this condition still remain obscure. Various reasons have been put forth as the predisposing factors, some of which are capsaicin, betel nut alkaloids, hypersensitivity, autoimmunity, genetic predisposition and perhaps malnutrition [2, 3].

Neoplasia is a multistage disease process, where a single cell can develop from an otherwise normal tissue into malignancy that can eventually destroy the very base. The series of cellular and molecular changes that occur through the development of cancers can be mediated by a diversity of endogenous and other free radicals which have long been known to be mutagenic. Further, these free radicals have more recently emerged as the mediators of the other phenotypic and genotypic changes that can lead from mutation to neoplasia. It has therefore been felt that free radicals may have a major contribution to the cancer development in humans [4, 5].
the Department of Dentistry, Hassan Institute of Medical Sciences, Hassan, Karnataka, India, who were clinically diagnosed as having oral submucous fibrosis.

The history and the clinical findings of each patient who were recorded as having submucous fibrosis was diagnosed by the presence of pallor of the oral mucous membrane, presence of palpable fibrous bands, lack of pliability of the oral mucous membrane and limited mouth opening. Consent was taken from each participant. The required investigations are carried out. All the patients were biopsied, both to confirm the diagnosis and to reveal any dysplasia which was present.

All the selected cases who were in the habit of chewing arecanuts, were advised to give up the habit and they were kept under observation for six weeks. No improvement was observed and later, the treatment was instituted.

Examination of the oral cavity was carried out under artificial light. Any abnormality and variation in the colour and the pliability of the oral mucosa was noted. The inter arch distance was measured in millimeters by using a graduated calipers kept between the incisal tips of the upper and lower central incisor with the mouth opening being maximum. The above measurement was taken bimonthly and it was recorded.

An incisional biopsy was performed in each patient at entry. The biopsies were taken from the buccal mucosa at the level of the cervical margin of the lower second molar, so as to avoid frictional keratosis at the level of the occlusal line.

In this study, the following parameters were evaluated:

1. **Clinical parameters:**
   a) Symptomatic relief in the form of absence of burning sensation in the mouth and spontaneous healing of the ulcers when present.
   b) Change in the colour of the mucosa and its texture.
   c) Improvement in mouth opening

2. **Histological parameters:**
   a) Surface mucosa showing non keratinization
   b) Rete-ridge formation with an increase in the epithelial layer
   c) Less infiltration of the inflammatory cells, mainly polymorphs, in the connective tissue.

**STUDY DESIGN**

After the clinical examination, investigation and biopsy confirmed the diagnosis as oral submucous fibrosis, each patient was given the commercially available anti-oxidant which was used in this study, LycoRed (Jagsonpal Pharmaceutical Company, New Delhi, India) capsule containing 100% natural lycopene with zinc, selenium and added phytonutrients. The zinc and selenium are essential for the proper assimilation of carotenes in our metabolism. This capsule was given orally, twice daily for 3 months, where each capsule contained 2000 µg of lycopene. The responding patients continued to take lycopene for an additional 3 months. The responses were assessed clinically and by bimonthly evaluation with examination and photographs. The increase in the mouth opening was measured by using graduated calipers.

All the biopsies were reviewed by a single pathologist at the entry level before the start of the treatment. After 3 months of treatment, a second biopsy was done as close as possible to the initial site and a histological evaluation was performed to confirm a clinical response.

Several factors should be considered when assessing these trials.

1. **Choice of the agent:** Although oral submucous fibrosis is pre-malignant, only chemo-preventive agents with minimal toxicity are justifiable for clinical testing (such as the antioxidant, lycopene).

2. **Assessment response:** It is difficult to apply oral photography consistently. The response can be best assessed, based on the examination of the condition by two examiners who can independently agree on a category with photographs as a helpful adjunct [Table/Fig-1, 2, 3, 4]. The increase in the mouth opening may be measured by using calipers [Table/Fig-5, 6, 7, 8].

3. **Evaluation of the histopathological response:** The comparison of the pre treatment biopsy with a biopsy specimen obtained after 3 to 6 months of the treatment was done. This is done to confirm a clinical response where a biopsy may show whether normalization has also occurred at the microscopic level [Table/Fig-9, 10 , 11, 12].
[Table/Fig-3]: Buccal Mucosa among group III Patients before treatment

[Table/Fig-4]: Buccal Mucosa among group III Patients after treatment

[Table/Fig-5]: Mouth opening among group II Patients before treatment

[Table/Fig-6]: Mouth opening among group II Patients after treatment

[Table/Fig-7]: Mouth opening among group III Patients before treatment

[Table/Fig-8]: Mouth opening among group III Patients after treatment
4. **Effect of habit changes on the response:** Patients who were in the habit of chewing betel nuts alone or in combination, were strictly advised to give up the habit and were kept under observation for six weeks. No improvement was observed and later, the treatment was initiated with lycopene.

5. **Study duration:** At least 3 months of treatment should be planned. The response can occur 3 to 6 months after the beginning of the treatment.

All the patients were grouped into four categories, based on the clinical and histological criteria (as described by Khanna J.N [10-12]):

**Group 1: Very early case**
**Clinical:** A burning sensation in the mouth was reported. Acute ulceration and recurrent stomatitis were seen. The mouth opening was normal.

**Histological:** A fine fibrillar collagen network interspersed with marked oedema. Young fibroblasts were seen, with abundant cytoplasm. Inflammatory cells mainly polymorphonuclear leucocytes were seen. The epithelium was essentially normal.

**Group 2: Early cases**
**Clinical:** The limitation of mouth opening was evident. The soft palate and the faucial pillars were the areas primarily affected. The buccal mucosa appeared to be mottled, with varying degrees of fibrosis. Histological: The results showed varying degrees of keratinisation, atrophy of the epithelium and juxta epithelial early hyalinization, decrease in fibroblasts and increase in fibrocytes. There was a dense infiltration of mixed inflammatory cells predominantly polymorphs.

**Group 3: Moderately advanced cases**
**Clinical:** Trismus was evident, ranging from 9mm to 15mm. The buccal mucosa was pale and was firmly attached to the underlying tissues. The vertical fibrous bands were palpated in the premolar area.
Histological: The results showed a marked reduction of the thickness of the epithelium and basal layer proliferation. The reteridges showed flattening and intercellular oedema in the focal areas. All the cases showed varying degrees of keratinization. The connective tissue showed moderate hyalinization with amorphous changes. Thick collagen bundles were seen, which were separated by slight oedema and they were infiltrated by lymphocytes and plasma cells, also occasionally by eosinophils.

**Group 4: Advanced cases with pre malignant and malignant changes**

Clinical: Trismus was severe. The mouth opening varied from 2-9 mm. The uvula was involved. The tonsils which were compressed could have been associated with leukoplakia and squamous cell carcinoma.

Histological: The collagen hyalization had a smooth sheath which eliminated all evidence of the individual bundles. Fibroblasts were markedly absent within the hyalinized zone. There was total loss of the epithelial rete pegs. There was mild to moderate atypia. Extensive degeneration of the muscle fibers was seen.

In our study, the groups 2 and 3 were selected: group 1 had normal mouth opening and in group 4, the disease was advanced, with malignant changes. Hence, the groups 1 and 4 were omitted.

**Results:** The responses are assessed clinically by bi-monthly evaluation with examination and photography. The responses were classified as:

1. **Complete**
   - When the colour of the mucosa turned from blanched white to normal pale pink.
   - When there was a definite improvement in the burning sensation, which reduced to an appreciable extent.
   - When there was an increase in the mouth opening which ranged from 2mm to 3mm.

2. **Partial**
   - When there was a partial improvement in the above said signs and symptoms and the mouth opening was increased by 0.4mm to 1mm.

3. **Stable**
   - When there was no response and no improvement.

4. **Disease progression**
   - When there was a progressive increase in the signs and symptoms in spite of having undergone the treatments.

**Patient characteristics:** A total of 12 eligible patients were included in this study, 3 females and 9 males. The age group of the study sample ranged from 18 to 52 years. Among the 12 patients, 6 were in the habit of pan chewing. Two patients were in the habit of smoking. Four patients were using pan with tobacco and slaked lime. No one was alcoholic. Four patients were using chillies in excess, whereas 8 patients had a normal intake of chillies. Seven patients were in group II (early cases). Their mouth opening ranged between 9mm to 14mm. Five patients were in group III (moderately advanced) and their mouth opening was between 9mm to 14mm [Table/Fig-13 and 14].

**Post Treatment Evaluation:** The number of cases in which the following improvement was seen after the treatment with lycopene, 2000 µg twice daily for 3 months, is shown in [Table/Fig-13 & 14]. The responses were assessed clinically and histologically as described below:

**IN GROUP II (EARLY CASES)**

**Clinical evaluation**

1. Disappearance of the burning sensation was noticed.
2. There is a change in the colour of the mucosa from pale blanching to normal pink [Table/Fig-1, 2].
3. Spontaneous healing of the ulcers was seen.
4. There was a definite improvement in the mouth opening, ranging from 1mm to 3mm [Table/Fig-5, 6, 13].

**Histological Evaluation**

1. Showed a mild degree of inflammatory infiltration in the connective tissue [Table/Fig-10].
2. The epithelial changes were more appreciable where in, they showed rete ridge formation with an increase in the epithelial layer.
3. In few cases, the surface mucosa almost showed a loss of keratinization.

**IN GROUP III (MODERATELY ADVANCED CASES)**

**Clinical Evaluation**

1. Disappearance of the burning sensation was observed.
2. Healing of the ulcers when present, was noticed.
3. There was a definite change in the colour of the mucosa from pale blanching to normal pink [Table/Fig-3, 4].
4. There was improvement in the mouth opening, ranging from 1mm to 3mm [Table/Fig-7, 8, 14].

**Histological Evaluation**

1. Showed a decrease in the keratinization layer.
2. An increase in the number of layers which reformed the rete ridges, with basal cell proliferation were seen [Table/Fig-12].

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Age/ Sex</th>
<th>Mouth opening</th>
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<th>Improvement</th>
<th>Response</th>
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<tr>
<td>2</td>
<td>20/ M</td>
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<td>2 mm</td>
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<td></td>
</tr>
<tr>
<td>3</td>
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<td>Complete</td>
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<tr>
<td>4</td>
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<td>26 mm</td>
<td>1 mm</td>
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<tr>
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<td>18 mm</td>
<td>2 mm</td>
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<tr>
<td>7</td>
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<td>36 mm</td>
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**Table/Fig-13: Post Treatment Evaluation Observed in Group II**

<table>
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<th>Post treatment</th>
<th>Improvement</th>
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<tr>
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<tr>
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<td>14 mm</td>
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**Table/Fig-14: Post treatment evaluation observed in group III**
RESPONSE DATA

Often, the first change which was noted was a decrease in the burning sensation and the appearance of the normal pink coloration of the mucosa. Subsequently, a definite reduction in the burning sensation and an increase in the mouth opening were noted. In a majority of the responding patients, the first signs of the response were visible within the second and third follow-up visits i.e. 4 patients at 1 month, 5 at 1½ months and 3 at 2 months. See [Table/Fig-15].

DISCUSSION AND CONCLUSION

Oral submucous fibrosis (OSMF) is a disease associated with the habit of betel nut chewing and it is characterized by extensive collagen deposition in the soft tissues of the mouth [8]. Various researchers, in their studies on the mutagenic properties of arecanuts have found that the constituents and the extracts of the nuts cause chromosomal aberrations and DNA damage[8]. In the review of literature, starting from Pay Master, 1959 to J.N. Khanna, 1995, every one is of the opinion that submucous fibrosis is a precancerous condition with a malignant transformation rate which varies from 3 to 7.6% [9]. The development of cancers can be mediated by a diversity of endogenous and environmental stimuli. Free radicals have long been known to be mutagenic. Further, these agents have more recently emerged as the mediators of the other phenotypic and genotypic changes that lead from mutation to neoplasia.

OSMF is an incurable disease. No treatment modality, either surgical or medical has been successful in completely eliminating the disease. In view of the strong relationship between oral cancer and pre cancerous lesions, chemo-prevention is said to be feasible and preferable than the conventional ones [3]. Hazardous treatments like the submucosal injections of steroids, hyaluranidase and non toxic and can be easily supplemented in the diet. It is clear from this, that a long term maintenance treatment is necessary, if there has to be an impact on oral cancer incidence. Lycopene may be more suitable for this purpose than the more toxic retinoids. Lycopene has been shown to inhibit various types of cancers and it has been shown to have potent benefits in oral pre malignant lesions such as oral leukoplakia, where it has been reported to modulate dysplastic changes. Another point in favour of the use of lycopene for the prevention of OSMF is that it is relatively non toxic and can be easily supplemented in the diet.

This was a short term study with a small sample size and the patients in this study were advised to give up the habit of pan and betel nut chewing. As we could not have a control group due to the limited number of patients, it was felt that a long term study with a larger sample size with all the above variables being taken into consideration would be necessary to get a clear picture about the utility of the drug.

REFERENCES


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<tbody>
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<tr>
<td>Complete response</td>
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<td>58.5%</td>
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<tr>
<td>Partial response</td>
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<td>41.5%</td>
</tr>
<tr>
<td>Stable</td>
<td>–</td>
<td>Nil</td>
</tr>
<tr>
<td>Progression</td>
<td>–</td>
<td>Nil</td>
</tr>
</tbody>
</table>

[Table/Fig-15]: Response Data
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