INTRODUCTION

ORAL SUBMUCOUS FIBROSIS (OSMF) is a chronic, progressive, scarring disease that predominantly affects the people of South-East Asian origin. The onset of the disease is insidious & chronic in nature. The prodromal symptoms includes burning sensation in the mouth when consuming spicy food, appearance of blisters especially on the palate, ulcerations or recurrent generalized inflammation of the oral mucosa, excessive salivation, defective gustatory sensation and dryness of the mouth. As the disease progresses, the oral mucosa become blanched and slightly opaque and white fibrous bands appear. With progressing fibrosis stiffening of certain areas of the mucosa occurs leading to difficulty in opening the mouth, inability to whistle or blow and difficult in swallowing. When the fibrosis involves the nasopharynx, the patient may experience referred pain to the ear and a nasal voice as one of the later signs in some patients. The pathogenesis of the disease is believed to be multifactorial. Various factors nutritional deficiency, chewing areca nut, genetic susceptibility, and autoimmunity and collagen disorders may be involved in the pathogenesis. However, the precise etiology of OSMF is still unknown. It has been reported that OSMF occurs mainly in persons who are habituated to chew areca nut or products containing arecanut along with other ingredients, in the recent years with the introduction of commercially available Gutkha and other areca nut products, the incidence of OSMF is increasing especially in the younger generation. This condition is of special concern to the dentist because there is no definite treatment modality available and permanent cure is not achievable. Considering this fact a continuous search for etiology is being done by many researchers. There has been a recent
interest in the role of copper as a possible etiological development of this disorder. Ma et al discovered increased lysyl oxidase activity in fibroblasts cultured from OSMF patients. Lysyl oxidase, an extra cellular copper enzyme is secreted by fibroblasts and initiates cross linking of collagen. Trivedi et al demonstrated that the copper content in areca nut products to be relatively high and that it is released in the mouth while chewing. The following study was carried out to determine the copper levels in serum of patients with oral submucous fibrosis and controls to throw light on role of copper in etiopathogenesis of OSMF.

MATERIALS AND METHODS
The study was carried out on 40 patients of oral submucous fibrosis who attended the Oral Diagnosis and Radiology department of Government Dental College and Hospital, Ahmedabad. Out of 40 patients selected, 34 were males and 6 were females. The male patients were between 18 to 45 years with an average of 27.58 years. The ages of females were between 20 to 60 years with an average of 36.66 years. All the patients had not received any kind of treatment for oral submucous fibrosis and none of them were suffering from any systemic diseases.

DIAGNOSTIC CRITERIA
O.S.M.F. was recognized solely on a clinical basis by following criteria.
1. Positive history of areca nut alone or in combination with tobacco chewing habit.
2. History of burning sensation of mouth on eating either normal / spicy food.
3. History of gradual restricted mouth opening.
4. Difficulty to protrude the tongue.
5. Clinically blanched oral mucosa.
6. Clinically palpable fibrous bands on the buccal mucosa and other areas of oral cavity.

After selecting the patient complete history of the patient, along with name, age, sex, relevant medical history, and serum copper level were recorded. The patient was examined under good light for better visibility. The intraoral examination was done with the help of mouth mirror and probe. 10 normal healthy controls with comparable age and sex were selected in the age range of 20 to 40 years. The controls had no pernicious habits of areca nut chewing or consumption of tobacco in any form and had normal healthy looking mucosa. They were not suffering from any systemic diseases. After taking the history of each patient 5ml blood were taken from each patient.

Serum Copper: About 5 ml of venous blood was collected from ante-cubital vein by aspirating in a pre-sterile disposable 5ml plastic syringe of 23 gauge needle. The collected blood was immediately transferred into an acid washed glass test tube and allowed to clot for about 30 minutes at room temperature without being disturbed. These test tubes with blood were then centrifuged at 3000 rpm for 15 minutes. With a disposable plastic pipette, serum was pipetted and transferred to a disposable vial. These vials were stored in the refrigerator, until the time of being analyzed.

Preparation of Diluent Solution: 60 ml of n – butyl alcohol was pipetted from a commercially available analytical grade ‘n’ butyl alcohol bottle. This was then diluted to 1000ml, in a volumetric flask of 1000ml using double distilled water.

Standard solution ; Pre-prepared atomic absorption standard solution of copper with a concentration of 1000mg/ml was used to prepare working solution.

Copper standard ; The working standards of copper solution were prepared at concentration of 1.2, 2.4, 3.6, 4.8 and 6.0 ppm respectively by adding diluents solution.

Atomic absorption spectrometry (AAS) ; The analysis of serum levels of copper was done by using atomic absorption spectrometry. (AAS – Model 3100, Perkin-Elmer)

RESULTS
Table1: Correlation of serum copper in oral submucous fibrosis with control subjects

<table>
<thead>
<tr>
<th>Serum Copper</th>
<th>Subject</th>
<th>Total</th>
<th>Mean (µg/Dl)</th>
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<tbody>
<tr>
<td>Osmf</td>
<td>40</td>
<td>93.05</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>92.13</td>
<td></td>
</tr>
</tbody>
</table>
In 40 OSMF patients mean serum copper level was 93.05µg/dl while in 10 controls was 92.13µg/dl which showed no effect on serum copper level in OSMF.

**DISCUSSION**

Oral submucous fibrosis is a well-recognized potentially malignant condition of the oral cavity. The disease is prevalent among the south Asian countries. Various factors have been implicated in the pathogenesis but there is strong epidemiological evidence associating the disease with the habit of areca nut\textsuperscript{4,8}.

Estimation of serum copper levels was done using Atomic absorption spectrophotometer in OSMF and control subjects. This method serves as a simple, sensitive and rapid method for analyzing trace elements with minimum contamination from extraneous metals. The mean value of serum copper levels in OSMF patients was 93.05µd/dl and in the control group was 92.13µd/dl. In the present study, we did not find any correlation mean serum copper level of OSMF patients and controls. This was in accordance with the finding of Trivedi et al.\textsuperscript{10} and Rajendran et al.\textsuperscript{9}.

This can be explained in several ways as effects of areca nut chewing more likely to be local within the areas of prolonged contact and small quantities that may be absorbed by swallowing the areca containing quid will be transported to the liver by the function of copper chaperones such as ceruloplasmin and transcuperin. These have the buffering capacity to maintain the serum copper at the steady state.

Significantly raised copper content in buccal epithelial cells of OSMF patients have important implication in the aetio-pathogenesis of this condition and its progression to malignancy because copper may induce DNA damage and alter the p53 conformation.\textsuperscript{9}

**REFERENCES**