MATERIAL & METHODS
Material and Methods

The present study was conducted on 104 patients attending out door of Department of Otolaryngology. M. L. B. Medical College & Hospital Jhansi, Bundelkhand region (U. P.). During the period of November 1998 to October 2000. Laboratory studies were done by various units of Department of Pathology. The special investigative process like blood biochemistry were done at Vardhman Medical Center, Jhansi. An effort was made to put light on etiology and pathological process of the disease.

Clinical Study:

Selection of patients was based upon the recognized sign and symptom of oral submucous fibrosis. Though the number of reported cases were such more but only those cases had been considered who were being followed up regularly with complete investigative process.

The patients under study, were subjected to thorough investigations. Detailed history was taken and physical examination with complete ENT Check up was done. The patients were kept on regular follow up and the improvement was assessed. Patients were divided into two groups:

1- Control group.
2- Study group.
Control Group:

These were those patients of oral submucous fibrosis, who were treated conservatively by mouth wash, analgesic, antioxidants and injection Hydrocortisone locally.

Study Group:

These patients were those who were treated by oral calcium and iron supplementation with mouth wash and analgesic.

Clinical History:

Special attention was done on following points while taking history of the patients –

- Duration of onset of problem.

- Complaints made by patient with chronological order i.e. inability to open mouth, intolerance to hot, spicy food and chillies. Difficulty to protrude tongue, pain and swelling at jaw joint and neck.

- Other associated complaints with main complaints like pain in the ears, recurrent stomatitis, nasal voice, nasal regurgitation, repeated ulceration of oral cavity, lose/decrease in taste sensation and inability to blow candle or to whistle were also interrogated.

- History of similar episodes in the past and if it was significant, what treatment, patient had taken, with assessment of result.
Personal History:

Since the personal history is much more significant, special attention was made to bring light on food habit i.e. patient being vegetarian, non-vegetarian or occasionally non-vegetarian, habit of using spices and chillies and the quantity & duration from which these substances being used. Use of any other similar irritating material associated with food habits.

Attention was paid during recording of the personal history of habituation and addictions. Chewing of tobacco with/without slaked lime was considered. Smoking habit and its pattern was also recorded. Use of betel nuts and betel chewing, with or without tobacco, was other area of special consideration. Use of pan masala and other similar commercial preparations, alcohol intake were also interrogated.

Hygiene of oral cavity and methods used by patients to clean teeth and oral cavity were also considered. Questions were asked about use of commercial tooth brushes, tooth paste/powders, use of plant sticks (like Neem or others), ash, charcoal and misi. Attention was paid on use of tooth powders mixed with tobacco (Gul). Questions were asked about how frequent these substances were used.
Personal History:

Since the personal history is much more significant, special attention was made to bring light on food habit i.e. patient being vegetarian, non-vegetarian or occasionally non-vegetarian, habit of using spices and chillies and the quantity & duration from which these substances being used. Use of any other similar irritating material associated with food habits.

Attention was paid during recording of the personal history of habituation and addictions. Chewing of tobacco with/without slaked lime was considered. Smoking habit and its pattern was also recorded. Use of betel nuts and betel chewing, with or without tobacco, was other area of special consideration. Use of pan masala and other similar commercial preparations, alcohol intake were also interrogated.

Hygiene of oral cavity and methods used by patients to clean teeth and oral cavity were also considered. Questions were asked about use of commercial tooth brushes, tooth paste/powders, use of plant sticks (like Neem or others), ash, charcoal and misi. Attention was paid on use of tooth powders mixed with tobacco (Gul). Questions were asked about how frequent these substances were used.
Social History:

Social status of the patients was also evaluated with related background of habitat (i.e. rural or urban). Classification of social status was made according to the classification suggested by Central Statistical Organization, Government of India (1962).

1. Upper Class - Principals, Academicians, Doctors, Lawyers, Engineers, Military Officer, Senior Executives & business proprietors.

2. Upper Middle Class - Junior executives, higher secondary school teachers, small business men.

3. Lower Middle Class - Clerks, primary school teachers, skilled labours like mechanics, electrician and formers.

4. Lower Class - Unskilled labours, peon, sweeper porters, shoemakers etc.

History of Previous Treatment:

Patients were asked about their previous treatment history, specific treatment if taken and improvement if obtained.
Other Associated Systemic Disease:

Detailed history was taken about other systemic disorders if present. Attention was paid about tuberculosis, diabetic, rheumatoid disorder, syphilis and lymphadenopathy.

Examination:

After completing detailed history, general and local examinations were performed. General examinations was including general condition of patients i.e. general outlook and general built and nutritional status of the patient. Patients were also examined for the occurrence of systemic disease like tuberculosis, diabetes lymphadenopathies, rheumatoid disorders and syphilis.

Local Examination:

Presence of any congenital anomaly, condition of angle of mouth, colour and condition of lips, general hygenic condition of oral cavity, colour of buccal mucosa, palate, tongue and uvula was noted. Concentration was made on the presence of fibrotic bands and its extension to the lateral wall of cheek, palate, uvula and tongue. Palpation of lateral wall of cheek was done bimanual by putting two fingers from inside and supported by two fingers or thumb of other hand from outside, to assess the thickness of fibrotic band. The patients were asked to protrude their tongue to observe ankyloglossia.
Progressive narrowing and inability to open mouth were assessed. It was done by measuring the distance between upper and lower incisors with the help of measuring caliper. The same technique was applied to evaluate the improvement during follow up of the cases and after the treatment. Severity of the trismus was classified into three different groups depending upon distance between upper and lower incisions.

1. Normal  
   1 - 1 distance  ≤ 3.5 cm.

2. Mild  
   1 - 1 distance  3.1 - 3.5 cm.

3. Moderate  
   1 - 1 distance  2.1 - 3.0 cm.

4. Severe  
   1 - 1 distance  < 2.0 cm.

Oral cavity was looked also for ulcerated lesions over tongue, cheek, palate, uvula, and lips. Status of teeth and gums were assessed for pyorrhea, cavities, sharp tooth and other associated lesions. Distribution of taste papillae and baldness of tongue were also noted. Ears were examined to rule out any possible disorder in relation to earache. Throat and none were also looked for any possibility of disease.

Observation of leukoplakia and its distributive pattern got a special consideration. Leukoplakic patches over palate, cheek, uvula, tongue and lips were thoroughly examined. Presence of growth weather benign or malignant in any part of the oral cavity were examined and recorded accordingly.
Clinical Stage of Disease:

Clinical staging of the disease was based on its clinical presentation and severity of the symptoms. Present staging of the disease was classified by De'Sa (1957).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Characteristic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I Early Stage</td>
<td>Burning sensation and irritation with hot spicy food, no clear-cut Fibrotic bands present, Oral Mucosa is blanched and loses its elasticity, No trismus, Slight restriction of mouth opening &amp; tongue protrusion normal.</td>
</tr>
<tr>
<td>Stage II Established Stage</td>
<td>Burning sensation and irritation with hot spicy food, Fibrotic bands Present, blanched opaque leather like mucosa, considerable restriction to mouth opening, slight restricted tongue protrusion, Oral hygiene poor.</td>
</tr>
<tr>
<td>Stage III Advanced</td>
<td>Burning sensation and irritation with hot spicy food, thick Fibrotic bands Present, blanched opaque leather like mucosa, very little mouth opening, restricted tongue protrusion, Oral hygiene very poor. Speech and eating very much impaired.</td>
</tr>
</tbody>
</table>
Clinical Chemistry Analyzer
Investigations:

Blood samples were collected for haematological, serological and biochemical investigations. Under hematological setup – total leukocytes count, differential leukocytes count and haemoglobin were evaluated. To evaluate haemoglobin, cyanomethaemoglobin method was employed. Under serological investigations – Erythrocyte Sedimentation Rate (ESR), demonstration of L. E. Cells, ASO and its titre were looked for. In biochemical observations total serum protein, Albumin/globulin ratio and VDRL test were performed. Total Serum calcium and serum iron were done as below mentioned method.

**SERUM CALCIUM ESTIMATION:**

**Principle:**

Colorimetric measurement with ortho-cresolphthalein complexion. The 8-hydroxyquinoline prevents mg 2+ from interference upto 4 mmol/L (100mg/L).

**Reagents Composition:**

**Reagent 1:**

Dimethylamine  
360 mmol/L

**Reagent 2:**

O-cresolphthalein complexion  
0.15 mmol/L

8-Hydroxyquinoline  
17.2 mmol/L

**Standard:**

Calcium  
10 mg/dL

100 mg/L

2.5 mmol/L.
Preparation and Satability of Working Reagent

Mix 1 volume of reagent 1 with 1 volume of reagent 2.

Stability : 20 hours at 2 – 8 °c

Reference Values

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum, Plasma</td>
<td>8.8</td>
<td>10.2</td>
<td>Mg/dL</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>102</td>
<td>Mg/L</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>2.55</td>
<td>Mmol/L</td>
</tr>
</tbody>
</table>

Procedure

1 ml of reagent is taken in 3 clean & dry test tubes. 10μL of distilled water is put in blank, 10μL Standard is put into standard test tube and then 10μL sample (Serum) is put into sample test tube. Mix well, and read the optical density (OD) after a 5 minute incubation. The final colour is stable for at least 1 hour.

Wavelength : 570 nm
Temperature : 37°C
Cuvette : 1 cm light path.
Read against reagent blank.

<table>
<thead>
<tr>
<th></th>
<th>BLANK</th>
<th>STANDARD</th>
<th>SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1mL</td>
<td>1 mL</td>
<td>1mL</td>
</tr>
<tr>
<td>Dist, Water</td>
<td>10μL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10μL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10μL</td>
</tr>
</tbody>
</table>

Mix and read optical density (OD) after a 5 minute incubation. The final colour is stable for at least 1 hour.

**Calculation:**

\[
\text{OD Sample} \quad \text{mg/dL} \quad n = 10 \\
\text{----------------- x n} \quad \text{mg/L} \quad n = 100 \\
\text{OD Standard} \quad \text{mmol/L} \quad n = 2.5
\]

\(n=\) standard concentration.

**Linearity**

UP TO 13.5 mg/dL (135 mg/L) (3.40 mmol/L).

**Serum Iron Estimation:**

**Principle**

Serum iron reacts with chromazurol B and cetytrimethyl ammonium bromide to form a coloured complex.

The intensity of the colour is proportional to the iron concentration.

**Reagents Composition**

Chromazurol B \( 0.2 \) mmol/L
Cetyltrimethyl ammonium bromide 2 mmol/L
Guanidine hydrochloride 3 mol/L
Acetate buffer, pH 5.0 45 mmol/L

Standard:
Iron 100 μg/dL
1 mg/L
17.9 μmol/L

Preparation and stability of working reagent

When stored at 250 C and protected from light, the reagents are stable until the expiry date stated on the label.

The reagent is ready for use.

Reference Value

50 - 168 μg/dL
0.5 - 1.68 mg/L
8.95 - 30 μmol/L

Procedure

1 ml of reagent is taken in 3 clean & dry test tubes. 50μL of distilled water is put in blank, 50μL Standard is put into standard test tube and then 50μL sample (Serum) is put into sample test tube. Mix well, and read the optical density (OD) after a 15 minute incubation. The final colour is stable for at least 1 hour.

Wavelength: 623 nm (620 – 640)
Temperature : 37 °C

Cuvette : 1 cm light path.

Read against reagent blank.

<table>
<thead>
<tr>
<th></th>
<th>BLANK</th>
<th>STANDARD</th>
<th>SAMPLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1mL</td>
<td>1 mL</td>
<td>1mL</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>50µL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>50µL</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>50µL</td>
</tr>
</tbody>
</table>

Mix well, and read the optical density (OD) after a 15-minute incubation. The final colour is stable for at least 1 hour.

**Calculation :**

\[
\text{OD Sample} \times n = 100
\]

\[
\text{OD Standard} \times n = 17.9
\]

\[n = \text{standard concentration.}\]

**Linearity**

UP TO 500 µg/dL (5 mg/L) (89.5 µmol/L).
Diagnosis:

The diagnosis of the disease was based mainly on the clinical presentation. The detailed history and complaint based physical examination was enough to establish the diagnosis.

TREATMENT

Control Group:

These were those patients of oral submucous fibrosis, who were treated conservatively by mouthwash, analgesic, antioxidants and injection hydrocortisone locally once weekly for 5 weeks.

Study Group:

These patients were those who were treated by oral calcium and iron supplementation with mouth wash and analgesic. Calcium was given by oral route as tab. calcium carbonate 500mg b.i.d. with vitamin D-400 i. u. / day. Iron was also given by oral route as tab. Iron (III) Hydroxide polymaltose complex eq. to Elemental Iron 100mg o.d. Iron & Calcium was given with time difference of more than two hours for better absorption because Iron and Calcium inhibit

Beside the medical treatment, all the patients were advised to stop taking tobacco in any form, stop smoking and to avoid any form of chronic irritation like alcohol, betel chewing, using pan masala, spicy food and chillies. Extraction of sharp tooth was advised, if present. They were also advised to clean the oral cavity properly
with adequate tooth powder/paste and tooth brush. Patients were asked to do antiseptic
gargle and mouth wash twice daily after each meal, to improve status of oral hygiene.

First Clinical Assessment:

The patients were examined on each visit to assess the improvement but through local and general examination and improvement was assessed after one month of the treatment. Improvement in the symptoms, previously presented by the patients were taken into consideration. Improvement in the colour of buccal mucosa, palate, cheek tongue and lips were observed along with improvement in the protrusion of tongue. The distance between upper and lower incisors was measured to assess the trismus. Minor symptoms, previously presented by the patients, were also examined the improvement.

Second Clinical Assessment:

Second Clinical assessment was done after completing second month of the treatment. Patients were examined for all of the symptoms, presented before. Emphasis was made on same line of observations on which first clinical assessment was done.
Third Clinical Assessment:

Third and final follow up was done after finishing the treatment (after 3 months). Patients were examined for all of the clinical findings. Relevant and significant investigations were repeated.

Management of Failure Cases:

Failure cases from each group were subjected to the combination of treatment which was consisting of intra oral hydrocortisone, hyaluronidase, systemic corticosteroid, antioxidants and multivitamin supplementation. The clinical assessments were made according to the previous assessments.

Malignant cases were managed either with surgical excision and radiotherapy or with radiotherapy alone, depending upon the need. Other supportive measures like surgical cutting of fibrous band, or condylectomy if necessary, were performed and the postoperative status of the patients was evaluated.