Abstract: Oral submucous fibrosis (OSMF) is a disabling, potentially malignant condition of the oral cavity. The aetiology of OSMF is multifactorial but remains obscure. Although arecanut is considered to be the most important causative agent, responses observed in individuals using arecanut vary in relation to quantity and duration. It is considered that an immunological process is responsible for the pathogenesis of disease. We correlated salivary immunoglobulin A (IgA), salivary immunoglobulin G (IgG) and serum immunoglobulin A (IgA), serum immunoglobulin G (IgG), levels by turbidometric immunoassay. We estimated the levels of total serum protein (TSP) and haemoglobin (Hb) to determine the role of nutritional deficiency. The study population comprised 30 cases of OSMF and 10 controls. Five milliliters of blood and 2 ml of saliva were collected. Quantitative analysis of serum and salivary IgG, IgA was done by turbidometric immunoassay. TSP and Hb were estimated by Biuret and cyanmethaemoglobin methods, respectively. All patients showed significant \((P < 0.01)\) increase in serum and salivary IgG, IgA levels as compared to controls. TSP patients showed significant \((P < 0.01)\) decrease as compared to controls. Results of Hb in patients were not significant. The estimation of immunoglobulin levels is important to support the concept of autoimmune basis. Estimation of TSP and Hb suggests that nutrition has a definite role in OSMF. (J Oral Sci 53, 97-102, 2011)
thick, fibrous bands in association with a blanched mucosa. Specific findings include reduction of the mouth opening, stiff and small tongue, blanched and leathery floor of the mouth, fibrotic and depigmented gingiva, rubbery soft palate with decreased mobility, blanched and atrophic tonsils, shrunken bud-like uvula, and sunken cheeks not commensurate with age or nutritional status.

Stage 3: Sequelae of OSMF are as follows
• Leukoplakia is found in more than 25% of individuals with OSMF
• Speech and hearing deficits may occur because of involvement of the tongue and the eustachian tubes (9)

Early diagnosis and treatment of OSMF is important due to its frequent association with leukoplakia and oral cancer (10).

The aetiology of OSMF is considered to be multifactorial (11), arecanut being the prime aetiology (12-15). Occurrence of OSMF in cases without any history of using irritants, in teenagers, idiopathic nature of the disease and various immunological changes have led many researchers to consider OSMF as an autoimmune disorder (6,16). Although immunoglobulin disorders cannot be prevented, if diagnosed early, they can be treated effectively. Hyperimmunoglobulinaemia is invariably associated with OSMF (16). Therefore, serum immunoglobulin levels are used as parameters to assess the status of humoral immunity (17). Our study aimed at estimation of serum IgG and IgG to assess the role of humoral immune response in OSMF.

Studies on serum immunoglobins are well hypothesized but the role of salivary immunoglobulins has been rarely explored. As irritants stay longer in saliva, they affect the oral cavity which is bathed in saliva (18). Thus, it is hypothesized that the local immune response also plays an important role in causation of OSMF (13). To explore this concept, our study aimed at estimation of salivary IgG and IgA and further determination of correlation of serum and salivary IgG and IgA to add on to the existing knowledge of the immune profile.

To explore the aetiological factors in OSMF, the concept of preconditioned mucosa has been debated for many years. Mucosa preconditioned due to nutritional deficiency leads to altered response to local irritants. Protein and haemoglobin (Hb) can be considered as broad indicators of anaemia and hence the status of nutrition. We have estimated the levels of Hb and total serum protein (TSP).

Reduction or elimination of the habit of arecanut chewing is the prime preventive measure. Various strategies have been tried in order to improve the condition, including nutritional support, physiotherapy, local and systemic corticosteroids and surgical management. Immuno-modulatory drugs have also been found to be effective (8). Thus, this study was undertaken to evaluate the immune profile along with estimation of TSP and Hb, which may open new avenues to understand the complex aetiological-pathogenesis of the disease and facilitate early diagnosis and treatment of the disease.

Materials and Methods
The present study was carried out after approval from Institutional ethical committee. Thirty cases of OSMF and 10 controls were selected from the Department of Oral Pathology, MDC and RC Indore. Detailed case history was recorded and patients were classified as very early, early, moderately advanced, advanced according to the criteria established by Khanna (9,13).

Khanna JN, Dave R (1995) classified oral submucous fibrosis into

Grade I: Very early or incipient stage
- Burning sensation; dryness of mouth; vesicles or ulcerations; irritation with spicy food; no change in mucosal color; no fibrous bands palpable; mouth opening 36-40 mm; tongue protrusion normal.

Grade II: Early stage
- Burning sensation and dryness of mouth, irritation with spicy food; oral mucosa blanched and loss of elasticity; no clear cut fibrotic band; slight restriction of mouth opening; tongue protrusion normal; mouth opening 26-35 mm.

Grade III: Moderately advanced stage
- Burning sensation and dryness of mouth; irritation with spicy food; blanched; opaque; leather-like mucosa’ vertical fibrotic bands on buccal mucosa making it stiff’ considerable restriction of mouth opening’ tongue protrusion not much affected’ difficulty in eating and speaking’ poor oral hygiene’ mouth opening 15-25 mm.

Grade IV: Advanced stage
- Burning sensation and dryness of mouth; irritation with spicy food; blanched; opaque; leather-like mucosa; thick fibrous bands on both buccal mucosa; retromolar area and at pterygomandibular raphe; very little mouth opening; restricted tongue protrusion; speech and eating very much impaired; very poor oral hygiene; mouth opening <15 mm.

The above classification considers clinical features including mouth opening. Patients having systemic diseases like diabetes, hypertension, liver disease, chronic infection,
and coexistent lesions were excluded from this study.

Five milliliters of blood was collected by venipuncture using 24-gauge needles, of which 2 ml was used for estimation of Hb by routine cyanmethaemoglobin method using a haemoglobin kit (Biolabs Diagnostics (I) Pvt. Ltd, Tarapur, Boisar, India). From remaining samples, blood serum was separated by centrifugation at 2500 rpm. For the collection of saliva, patients were asked to rinse their mouth with distilled water and incline the head forward. Two milliliters of mixed, unstimulated saliva that pooled in the mouth were collected in a clean sterile glass tube. The sample was centrifuged at 2,500 rpm for 15 min. The separated supernatant was used.

Serum and salivary IgG and IgA were quantified by using a diagnostic kit (Quantia IgG and IgA turbidometric immunoassay for estimation of immunoglobulin IgG and IgA, Tulip diagnostics [P] Ltd., Goa, India). For estimation of serum or salivary IgG, 500 µl of quantia IgG activation buffer R1 was taken in a clean cuvette. Serum sample was diluted in the ratio of 1:5 with normal saline. Either 5 µl of diluted serum sample or 500 µl saliva sample was added to R1. After incubation of 10 min, 50 µl of R2 was added; i.e., Quantia IgG antihuman IgG reagent to sample and reading was recorded at wavelength 340 nm at 37°C. Finally, the result was multiplied by 5 for estimation of serum IgG and divided by 100 for estimation of salivary IgG.

For estimation of serum IgA, a similar procedure was used except that instead of 5 µl, 10 µl of the diluted serum sample was added to R1. For estimation of salivary IgA, either 10 µl/20 µl/50 µl of saliva sample was added to R1. Result was calculated accordingly. For estimation of TSP, the Lyphozyme Total Protein diagnostic kit (Beacon Diagnostics Pvt. Ltd., Navsari, India) was used and estimation was done by the Biuret method.

Results

Data were tabulated and statistically analysed using the t-test. Karl Pearson correlation test was used to correlate the relationship between different parameters. Comparison of different parameters between controls and grades of OSMF was done by t-test.

All the patients of OSMF showed significant (P < 0.01) increase in serum and salivary IgG, IgA levels as compared to controls. TSP showed a significant (P < 0.01) decrease when compared with controls. Hb did not show a significant (P < 0.01) decrease as compared to control subjects (Table 1).

Serum IgG showed significant (P < 0.01) increase with an increase in clinical grades of OSMF. Serum IgA showed significant (P < 0.01) decrease with an increase in clinical grades of OSMF. Salivary IgG and IgA, although not significant (Table 2).

Serum IgG showed significant (P < 0.01) increase with an increase in clinical grades of OSMF. Serum IgA showed significant (P < 0.01) decrease with an increase in clinical grades of OSMF. Salivary IgG and IgA showed no significant increase or decrease as grades of OSMF increased. As grades of OSMF increased, TSP did not progressively decrease. Hb showed progressive decrease with an increase in grades of OSMF, but it did not reach statistical significance (Table 3).
Discussion

The precancerous nature of OSMF has been emphasized by several authors and often there seems to be a wide field of cancerization in OSMF. Since several factors have been associated with the aetiology, there is a need to explore the aetiology of OSMF (11).

Most of the patients in our study were aged 20-40 years with the mean age being 34.30 years showing male predominance. These results were in agreement with previous studies, probably due to social encounter, economic liberty, popularity of refined arecanut products and easy availability of the product (4,19-23).

Several theories have been put forth to explain the aetiology of OSMF, such as use of betel nut, tobacco and allergy to chillis. However, cases with none of these factors and occurrence of OSMF in young adults have led to postulation of immune mechanism as the basis of OSMF (12). Response to antigen is prevalent and immunity plays a significant role in OSMF. This study was performed to determine this association.

Immunoglobulins are a heterogenous group of proteins having three major classes of immunoglobulin G, A, M designated as IgG, IgA, IgM. Raised serum IgG levels have been reported by Gupta et al., Caniff et al., and Shah et al. (5,24). The role of active immune phenomenon in OSMF is supported by accelerated body defence and the polyclonal nature of the disease, incidence of autoantibodies and involvement of the DR Locus, altered antigen leading to defective lymphocyte function and hyperactivity of B cells leading to hyperglobulinaemia (5,24-26).

Increased levels of serum IgA observed in the present study are in accordance with results reported by Gupta et al., Rajendran et al., and Shah, but in contrast with those reported by Canniff, Chaturvedi, Chaturvedi and Sharma. Though it is difficult to explain the reason for differences in IgA in various studies, results do implicate hyperglobulinaemia, imbalance in immunoregulation and alteration in local tissue architecture (5,19,20,24,25,27).

The role of serum immunoglobulins has been well hypothesized, but little is known about role of saliva, as saliva is not a popular body fluid to investigate. Still many scientists appreciate the miracle of saliva. Salivary immunoglobulin plays an important role in OSMF since irritants stay longer in saliva (28). Abrol found results similar for salivary IgG but contrasting result for IgA (18). Phatak found normal levels of salivary immunoglobulins. (15) Weyhmeyer et al. and Tupkari and Challacombe support the view that IgA is the predominant immunoglobulin secreted into external secretions including saliva and tears. Thus, increase in salivary IgA is due to increased local infection, increased antigenic inflammatory stimulus, increased local synthesis and local host reaction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control patient</th>
<th>Grade II OSMF</th>
<th>Grade III OSMF</th>
<th>Grade IV OSMF</th>
</tr>
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<tr>
<td>Serum IgG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (1189mg %)</td>
<td>1837</td>
<td>1872</td>
<td>1944</td>
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<tr>
<td>t’ value</td>
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<td>12.63 (2.18)</td>
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</tr>
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<td>Probability and Significance</td>
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<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
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<tr>
<td>Mean (154mg %)</td>
<td>376</td>
<td>364</td>
<td>325</td>
<td></td>
</tr>
<tr>
<td>t’ value</td>
<td>6.75 (2.44)</td>
<td>7.77 (2.18)</td>
<td>8.29 (2.44)</td>
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<tr>
<td>Probability and Significance</td>
<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
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</tr>
<tr>
<td>Serum IgA</td>
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<tr>
<td>Mean (0.057mg %)</td>
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<td>0.17</td>
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<tr>
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<td>3.25 (2.18)</td>
<td>2.21 (2.44)</td>
<td></td>
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<tr>
<td>Probability and Significance</td>
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<td>0.003 (Sig)</td>
<td>0.041 (NS)</td>
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<td>Salivary IgG</td>
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<td>22.38</td>
<td>30.75</td>
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<tr>
<td>t’ value</td>
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<td>4.12 (2.18)</td>
<td>2.51 (2.44)</td>
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<td>Probability and Significance</td>
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<td>0.000 (HS)</td>
<td>2.51 (Sig)</td>
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<tr>
<td>Probability and Significance</td>
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<td>0.000 (HS)</td>
<td>0.000 (HS)</td>
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<td>Hemoglobin</td>
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<td>1.77 (2.44)</td>
<td></td>
</tr>
<tr>
<td>Probability and Significance</td>
<td>0.636 (NS)</td>
<td>0.207 (NS)</td>
<td>0.095 (NS)</td>
<td></td>
</tr>
</tbody>
</table>

NS = Non-significant, HS = Highly significant, Sig = Significant
against the presence of disease.

Kin Kella Heiner, in a study done on oral mucosal disease, suggested that higher levels of salivary IgG may be due to increase in serum IgG which passes from vascular and extravascular compartment into saliva. Thus, IgG in saliva may originate from serum and be transported by passive transmucosal diffusion (26). However, in our study, serum and salivary IgG were not significantly correlated, possibly because of trace amounts of salivary IgG.

Serum and salivary IgA are positively correlated, although not significantly. This may be because of back diffusion from salivary IgA into serum as in accordance with Thompson and Asqueth (29). However, some authors state that this may also be due to increased local synthesis or due to local host reaction as salivary IgA is biochemically different from serum IgA.

Proteins are physically and functionally complex macromolecules that perform multiple roles (30). Levels of protein, Hb, vitamins B complex etc in OSMF are important factors which suggest the role of nutrition in OSMF. A significant decrease in TSP and an insignificant decrease in Hb in the present study partially support the view of Sirsat and Khanolkar who suggested that in mucosa preconditioned by nutritional deficiency in the form of protein, Hb has more pronounced response to capsaicin (18). Protein and Hb are considered broad indicators of nutrition.

The Seed and Soil theory put forth by Ramanathan states that OSMF is an Asian version of sideropenic dysphagia, where iron deficiency leads to mucosal susceptibility to irritants such as betal nut (31).

Hb levels were estimated to correlate anaemia with OSMF as anaemia has been found to be common in OSMF. The nonsignificant results may be explained by factors like nonconsumption of alcohol, middle to high socioeconomic status of patients and nutritionally non-deprived patients. In our study, we observed that as the grade of OSMF increased, Hb levels were decreased. This partly supported the view of Lavina Taneja (23) that OSMF is a collagen disorder. Decreased iron levels are due to fibrosis which requires iron for deposition of collagen. The initial symptoms make consumption of solid food an unpalatable affair leading to further poor food intake and anaemia; thus, this vicious cycle goes on leading to further iron deficiency. Thus, we conclude that nutritional deficiency could affect rather than cause oral sub mucous fibrosis.

Immunology has a definite role in OSMF and thus, there is imbalance of immunoregulation as well as alteration of local tissue architecture. Decreased TSP is a result of host response and Hb as an indicator of nutritional status plays an important role. However, further studies are needed to definitely state its cause or effect relationship. Immunological follow-up of OSMF patients will be beneficial for early detection of the transformation process of OSMF to oral carcinoma.

References

15. Murthi PR, Gupta PC, Bhonsle RB, Daftary DK, Mehta FS, Pindborg JJ (1990) Effect on the incidence of oral submucous fibrosis of intervention in the area...