Comparative evaluation of serum and salivary immunoglobulin G and A levels with total serum protein in oral submucous fibrosis patients: A case control study

M. Kandasamy, N. Jaisanghar¹, Ravi David Austin², Kumar Chandan Srivastava³, G. Sai Anusuya⁴, N. Anisa⁵

ABSTRACT

Aim and Objective: The objective of this study is to estimate and compare the serum and salivary immunoglobulin G and A (IgG, IgA) levels in various stages of oral submucous fibrosis (OSMF) patients and relate it to total serum protein (TSP) and hemoglobin (Hb) levels. Materials and Methods: The sample for the present study comprised a total of 20 healthy controls, 20 OSMF patients. About 5 ml 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 cyanmethemoglobin methods, respectively. Results: Serum and salivary IgG and IgA levels were statistically significantly increased (P < 0.001) in OSMF patients when compared to controls. Also serum and salivary IgG and IgA levels showed significantly increased (P < 0.01) in all the three staging of OSMF when compared to control group. Hb levels and TSP levels were significantly decreased (P < 0.001) in OSMF patients when compared to controls. One-way ANOVA, Pearson’s correlation, and unpaired t-test were used for statistical analysis. Conclusion: The elevated levels of IgG and IgA are also in favor of polygammapathy, which are nonspecific and nondiagnostic objective reflections of an underlying disease. Decreased TSP is a result of host response and Hb, acts as an indicator of nutritional status plays an important role. It is also observed from the present study that the severity of OSMF was directly proportional to the estimated elevated levels of the major IgG and IgA. A need is also felt for the knowledge of immunoprofile estimation in etiology and pathogenesis that would prove a great asset in the proper assessment of this condition.

KEY WORDS: Hemoglobin, immunoglobulin A, immunoglobulin G, oral squamous cell carcinoma, oral submucous fibrosis, total serum protein

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Kandasamy M, Jaisanghar N, Austin RD, Srivastava KC, Anusuya GS, Anisa N. Comparative evaluation of serum and salivary immunoglobulin G and A levels with total serum protein in oral submucous fibrosis patients: A case control study. J Pharm Bioall Sci 2016;8:S126-32.
A wide spectrum of oral mucosal lesions can be encountered by dental and medical practitioners in their routine practice. A precise diagnosis is needed as these lesions may vary in nature from simple to life threatening ones.

Oral submucous fibrosis (OSMF) is one such lesion, which though is easy to diagnose but difficult to manage. The disease was first described by Schwartz in the year 1952 and was named “Atrophia idiopathica (tropicum) mucosum oris.” This was followed by the first description of this condition in India in 1953 in quick succession by Lal and Joshi.[1]

Pindborg on the basis of clinical and histopathological findings defines OSMF as “an insidious chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a Juxta-epithelial inflammatory reaction followed by a fibro elastic change of the lamina propria with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat.”[2,3]

It is a chronic progressive disorder and its clinical presentation varies with the stage of the disease at the point of clinical detection.[4]

It is estimated that 2.5 million people are affected worldwide over a 10-year period. OSMF also has a statistically significant mortality rate because it is a precursor to oral squamous cell carcinoma seen in 7.6% of the cases.[5]

The etiology of OSMF is considered to be multifactorial,[6] area nut being the prime etiology.[7,8]

The occurrence of OSMF in cases without any history of etiological agents and various immunological changes have led many researchers to consider OSMF as an autoimmune disorder.[9,10]

As the disease produces changes localized to the oral cavity, saliva does not play a direct role in the pathogenesis of OSMF, but it can act as vehicle or play an indirect role. Thus, it is reasonable to assume that saliva may inhibit certain features, which can serve as indicators of the disease.[11]

With the increasing number of literature on immunoglobulin disorders and a wide variety of symptoms and diseases with which they can be associated. Studies conducted on serum and salivary immunoglobulins G and A (IgG and IgA) profile of patients with OSMF have shown conflicting results.[12]

From the review, it is noticed that only limited work has been done on pertaining to the role of OSMF. Therefore, there is a need for the present study to assess the estimation of serum and salivary IgG and IgA in OSMF patients.

**Materials and Methods**

The study was conducted at Rajah Muthiah Dental College and Hospital, Annamalai University. The study population comprised 20 cases of OSMF and 20 controls.

Group A includes twenty patients of OSMF diagnosed based on the history and clinical findings. Group B - 20 age, sex, habit, and socioeconomic status-matched controls were included in the study. A formal ethical clearance to conduct the study was given by the Ethical Committee of the college. A formal informed written consent was obtained from the individuals included in the study.

A detailed case history was recorded for all patients with special reference to their habits (chewing of betel nut, pan parag), its nature, duration, and frequency of use. All patients were subjected to a thorough general physical and oral clinical examination, and details were recorded on standard Proforma.

Patients who are physically healthy and are well oriented in time, space, and as a person, patients who satisfy the characteristic clinical features of OSMF, patients who are not taking any medication for their condition were included in this study.

Patients with OSMF and any past or present systemic disease (diabetes, liver disorders, hypertension, kidney disease, pregnancy) were excluded from this study. Clinical staging of OSMF was based on the classification by Khanna and Andrade.[13]

Khanna and Andrade developed a group classification system for the surgical management of OSMF. Group I: Very early cases: Common symptom is burning sensation in the mouth, acute ulceration, and recurrent stomatitis and not associated with mouth opening limitation.

Group II: Early cases – Buccal mucosa appears mottled and marble such as widespread sheets of fibrosis palpable, interincisal distance of 26–35 mm.

Group III: Moderately advanced cases – Trismus, interincisal distance of 15–25 mm, buccal mucosa appeal’s pale firmly attached to underlying tissues, atrophy of vermilion border, vertical fibrous bands palpable at the soft palate, pterygomandibular raphe, and anterior faucial pillars.

Group IVA: Advanced cases – severe trismus, interincisal distance of <15 mm, thickened faucial pillars, shrunken uvula, restricted tongue movement, presence of circular band around entire lip and mouth.

Group IVB: Advanced cases – presence of hyperkeratotic leukoplakia and/or squamous cell carcinoma.

**Collection of blood sample**

About 5 ml of blood was collected by venipuncture using 24-gauge needles, of which 2 ml was used for the estimation of hemoglobin (Hb) by routine cyanmethaemoglobin method. Samples were collected in ethylenediaminetetraacetic acid bottles. The samples were centrifuged for 15 min at 2500 rpm, and plasma was separated from the cellular components using a plastic Pasteur pipette.
Collection of saliva sample

Whole nonstimulated saliva was collected by asking participants to spit into a graduated universal bottle for a period of 10 min. This was immediately transferred to sterilized tubes and frozen. The samples were stored at −20°C until the time for immunoglobulin measurement. The sample was centrifuged at 2500 rpm for 15 min. The separated supernatant was used.

Turbidimetric immunoassay method

Quantia IgG is a turbidimetric immunoassay for the detection of IgG in human serum and is based on the principle of agglutination reaction. The test specimen is mixed with the activation buffer (R1) and then with antihuman IgG reagent (R2) and allowed to react. For the 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 salivary 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. The extent of turbidity corresponds to the concentration of IgG in the test specimen.

Finally, the result was multiplied by 5 for the 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 total serum protein (TSP), the Lyphozyme Total Protein diagnostic kit was used and estimation was done by the Buret method.

Statistical analysis

Statistical analysis of the data was carried out with SPSS for Windows 9.0 software (SPSS, Inc., Chicago, IL, USA). One-way ANOVA, un paired t-test, and Pearson’s correlation were used for statistical analysis.

Results

About 40% of the patients included in this study falls under the age group of <30 years in Group (A) with a mean range of 37.10 ± 12.19. 35% of the patients included in this study falls under the age group of 41–50 years in Group (B) with a mean range of 39.90 ± 1.65% of the patients included in this study in Group (A) were male. About 55% of the patients included in this study in Group (B) were male. Around 60% of the patients included in Group (A) having duration of habit more than 5 years with a mean range of 6.65 ± 4.60. Nearly, 70% of the patients included in Group (A) having frequency of habit more than 5 times per day with a mean range of 5.20 ± 3.44.

On performing group comparison by using un paired t-test, serum and salivary (IgG and IgA) levels in Group A were found to be significantly increased when compared with Group B. TSP and Hb levels in Group A were found to be statistically significantly decreased when compared with Group B.

On performing one-way ANOVA test, serum IgA, IgG, salivary IgA, IgG, TSP, Hb were found to be statistically significantly increased with the progression of stagings.

On performing Pearson’s correlation, serum IgG and salivary IgG showed a positive correlation but not statistically significant. Serum IgA and salivary IgG showed a positive correlation but not statistically significant.

Discussion

OSMF is a chronic progressive condition of the oral mucosa that has been largely reported among Indians and to lesser extent in other Asian people. 

Till date, the disease is one of the most poorly understood and unsatisfactorily treated. This is mainly due to fact that the etiology of the disease is not fully understood and the disease is progressive in nature. Several factors such as areca nut chewing, nutritional deficiency states, chilli consumption, genetic susceptibility, autoimmunity, and collagen disorders have been suggested to be involved in the pathogenesis of this condition. Based on the clinical and epidemiological studies, areca nut chewing is considered as the most in criminating causative agent. However, from the data currently available on OSMF, it appears quite clear that the disease is multifactorial because it is a precursor to oral cancer, particularly squamous cell carcinoma.

However, cases associated with none of these factors and occurrence of OSMF in young adults has led to postulation of immune mechanism as the basis of OSMF. Varied Response to antigen is prevalent and immunity plays a significant role in OSMF.

The role of active immune phenomenon in OSMF is supported by accelerated and altered body defense. Incidence of auto antibodies and defective lymphocyte function has been observed from earlier studies.

Many investigators suggest an autoimmune basis for OSMF. It may be because of the presence of human leucocyte antigen A10 and DR3, DR7 antigens found in OSMF patients. Also increased autoantibodies directed toward the gastric parietal cells, thyroid gland, antinuclear antibodies, antismooth muscle antibodies has been seen in OSMF.

Humoral immunity may also play a role as there is an increase in circulating immune complexes and increased serum levels of IgG and IgA have been found in OSMF patients.

Alterations in the levels of serum immunoglobulins have been described in various disease states and in some patients this may be a helpful diagnostic aid.

Saliva is well known for its highly protective functions against deleterious agents such as microorganisms, toxins, and various
oxidants. There is a growing interest in saliva as a diagnostic fluid due to its relatively simple and noninvasive collection. Such tests can be a faster, cheaper, and potentially safer diagnostic method than blood sampling.

It is more likely that studying the immunologic alterations of saliva and serum in patients with OSMF will lead to additional knowledge of its etiology, pathogenesis, and management from a new dimension.

In our study, 40% of patients were in the age group of 15–30 years [Table 1]. Earlier Sirsat et al. reported majority of OSMF cases belonged to the age group of 20-40 years of age. Sinor et al. reported 79 per cent of the OSMF cases were under the age of 35 years and maximum numbers of cases were in 25-44 years of age group.

This study indicates that more of the younger people are suffering from OSMF. This may be due to easy availability, attractive packaging, and advertisements. During this age, they indulge in various chewing habits such as betel nut, betel quid, and pan masala due to stress.

In Our present study majority of the patients showed a male predominance [Table 1]. Similar result was reported by Vanaja Reddy et al.

In a study from Durban, south africa. A distinct female predominance was identified, with a male to female ratio of 1:1.3 Van Wyk CW et al. This was later confirmed by Aziz. Male predominance in our study can be related to easy accessibility of areca nut and its products to males than to the females.

In our study, majority of the patients (70%) showed an average frequency of habit 5 times per day [Table 1]. Patients with the habit frequency of 5–10 times in a day had the maximum number of lesions. Patients with a combination of habits had fewer lesions, the possible reason being reduced time of contact or exposure to each individual habit. The duration and frequency of habits has a significant effect on the development of oral lesions which can be noted in the findings. Aruna et al.

In our study majority of patients (60%) who chewed areca nut for duration of more than 5 years [Table 1] developed OSMF. These results were consistent with that proposed by Shah et al. According to whom, the risks increase with the duration of habit.

In our study, we attempted staging for Group A subjects. In Group A, 10 subjects fall in Stage II [Table 1]. These findings are of great concern because younger individuals are at greater risk as it has been well established that OSMF is a premalignant condition of the oral mucosa.

| Characteristics                        | OSMF patient (A) | Controls (B) |
|----------------------------------------|-----------------|-------------|
| Age                                    |                 |             |
| ≥30                                    | 8               | 5           |
| 31-40                                  | 3               | 5           |
| 41-50                                  | 7               | 7           |
| >50                                    | 2               | 3           |
| Total                                  | 20              | 20          |
| Gender (%)                             |                 |             |
| Male                                   | 13              | 11          |
| Female                                 | 7               | 9           |
| Total                                  | 20              | 20          |
| Duration of habit of OSMF patients (years) |             |             |
| ≥5                                     | 12              | 20          |
| 6-10                                   | 5               | 5           |
| >10                                    | 3               | 3           |
| Total                                  | 20              | 20          |
| Frequency of habit of OSMF patients (times) |             |             |
| ≥5                                     | 14              | 10          |
| 6-10                                   | 4               | 4           |
| >10                                    | 2               | 2           |
| Total                                  | 20              | 20          |
| Staging of OSMF patients (%)           |                 |             |
| I                                      | 3               | 10          |
| II                                     | 10              | 50          |
| III                                    | 7               | 35          |
| Total                                  | 20              | 100         |

OSMF: Oral submucous fibrosis, SD: Standard deviation
Serum immunoglobulin levels, which are used as the parameters to study on humoral immunity, still continue to be an area of intensive investigative research. The changes in serum immunoglobulin levels may be initiated much before the actual clinical symptoms appear.

In this study, the mean serum IgG and IgA level among OSMF subjects were statistically significantly increased ($P < 0.001$) as compared to the control group [Table 2], which is similar to the study done by Shah.[27] Raised globulin levels are indicative of immunological disorders. The increased levels of serum IgG, IgA fractions of immunoglobulins in the experimental group of patients highlight the active role of immune phenomenon at work in OSMF.

Circulating auto antibodies are also present in some cases of OSMF.

In this study, the mean serum IgG levels were statistically significantly ($P < 0.01$) elevated with the increased stagings of OSMF [Table 3]. The above observations were similar to those reported by Shah.[27]

In this study, the mean serum IgA levels were statistically significantly ($P < 0.01$) elevated with the increased stagings of OSMF [Table 3]. On the contrary, Chatuvedi[28] reported a statistically nonsignificant decrease serum IgA levels with increasing stagings of OSMF.

In this study, the mean Salivary IgG and IgA levels among OSMF subjects were statistically significantly increased ($P < 0.001$) as compared to the control group [Table 2], which is similar to a study done by Prashanth et al.[12]

Sato[29] reported a statistically significant ($P < 0.001$) raise in salivary IgG levels in various mucosal diseases such as lichen planus, leukoplakia, and squamous cell carcinoma.

The significant increase in levels of these major immunoglobulins is also suggestive of accelerated body defense among such patients.

The elevated levels of IgG, IgA are also in favor of polygammopathy, which are nonspecific, nondiagnostic objective reflections of an underlying disease.

Increase in immunoglobulin levels is typically associated with three main chronic disease classes, those affecting the liver, collagen disorders, and chronic infections.

Thus, increase in salivary IgA and IgG is due to increased local infection, antigenic inflammatory stimulus, local synthesis, and local host reaction against the presence of disease.

Proctor and Carpenter[30] found an increase in S-IgA in the affected individuals. This is suggestive of reactive phenomena trying to limit the disease. S-IgA which forms the major surface defense mechanism increases and tries to limit the disease progression.

In this study, the mean salivary IgG levels were significantly ($P < 0.01$) elevated with the increasing stagings of OSMF [Table 3]. This indicates the presence of active phenomena trying to halt the disease progress by building a barrier and preventing further changes Prashanth et al.[12]

In this study, Hb levels were used to correlate anemia with OSMF. In our study, we observed that the mean Hb levels among OSMF subjects were significantly decreased ($P < 0.001$) when compared to control group [Table 2].

Low levels of Hb and serum iron are suggestive of iron deficiency anemia. In a study by Derossi and Raghavendra,[31] it is found that iron deficiency anemia in patients with OSMF could be related to the precancerous nature of this condition. Further lack of iron in tissues causes improper vascular channel formation resulting in decreased vascularity. This leads to a derangement in the inflammatory and reparative response of the lamina propria resulting in defective healing and scarring. Thus, the cumulative effect of these initiating and promoting factors leads to further fibrosis, which is a significant feature of OSMF. Taneja et al.[32]

Levels of protein, Hb, Vitamins B complex, etc., are important factors which substantiate the role of nutrition in OSMF. In similar context, Taneja et al.[32] stated that the mucosa is preconceived by such nutritional deficiency. Also in the current study, a statistically significant ($P < 0.001$) decrease of TSP in OSMF patients when compared to control group [Table 2] in observed. Similar observations were made by Cox and Walker,[14] that the mucosa is preconceived by nutritional deficiency Taneja et al.[32]

Table 2: Group comparison of variables of oral submucous fibrosis and controls

| Parameters study group | Expressed in mean and SD |
|------------------------|--------------------------|
|                        | Salivary IgG (mg%) | Salivary IgA (mg%) | Serum IgG (mg%) | Serum IgA (mg%) | Total serum protein (g%) | Hemoglobin (g%) |
| OSMF (Group A)         | 0.265±0.148           | 23.56±9.74        | 1611.80±259.00 | 214.60±56.84   | 5.72±0.80               | 9.99±1.65     |
| Controls (Group B)     | 0.0725±0.0272         | 13.25±2.26        | 1168.30±80.83  | 128.45±30.49   | 7.72±1.01               | 12.07±2.01    |
| t                      | 5.378                 | 4.479             | 7.964          | 6.004          | −12.982                 | −2.75         |
| P                      | 0.000***              | 0.000***          | 0.000***       | 0.000***       | 0.000***                | 0.006***      |

Unpaired t-test; ***VHS: Very high statistically significant, $P<0.001$. SD: Standard deviation, OSMF: Oral submucous fibrosis, Ig: Immunoglobulin

* Kandasamy, et al.: Immunoglobulin levels in OSMF

* S130 Journal of Pharmacy and Bioallied Sciences October 2016 Vol 8 Supplement 1
In this study, serum and salivary IgG showed positive correlation, but this was not significant ($P < 0.05$). Serum and salivary IgA showed a positive correlation; however, it was not statistically significant ($P < 0.05$) [Table 4]. This may be because of back diffusion from salivary IgA into serum. However, this may also be due to increased local synthesis or due to local host reaction as salivary IgA is biochemically different from serum IgA Lee.

Hb showed negative correlation with serum and salivary IgG, IgA, and TSP although it was not statistically significant ($P < 0.05$).

Considering the uncertain etiology, this condition should be viewed from a broader perspective and all the avenues for periodic immunological follow-up in the patients with OSMF could be an excellent model for studying genetic, environmental, immunologic, nutritional interactions in disease pathogenesis. Immunological follow-up of OSMF patients will be beneficial for early detection of the transformation process of OSMF to oral carcinoma.

### Conclusion

OSMF was more prevalent in males and the mean age was statistically significantly less compared to females. Serum and salivary IgG and IgA levels have been found to be statistically significantly increased ($P < 0.001$) in OSMF patients when compared to the control group. Moreover, serum and salivary IgG and IgA levels showed a statistically significant increase ($P < 0.01$) in the three stagings of OSMF, thus indicating some role in the pathogenesis of OSMF.

The increased levels of the serum IgG and IgA fractions of immunoglobulins in the study group of patients highlight the role of active immune phenomenon at work in OSMF. Decreased TSP and Hb can serve as an indicator of nutritional status, which plays an important role in OSMF.

A need is also felt for the knowledge of immunoprofile estimation in etiology and pathogenesis of OSMF that would prove a great asset in the proper assessment of this condition, which helps in delivering the appropriate treatment procedure. The transformation of OSMF to OSCC can be identified even in the early stage, the benefit of which can be achieved by periodic immunological follow-up in the patients with OSMF.

### Financial support and sponsorship

Nil.

---

**Table 3: Comparison of parameters with clinical staging of oral submucous fibrosis**

| Variables          | Staging | $n$ | Mean   | SD    | $F$ value | $P$ value |
|--------------------|---------|-----|--------|-------|-----------|-----------|
| Salivary IgG (mg%) | I       | 3   | 0.087  | 0.0255| 9.414     | 0.002***  |
|                    | II      | 10  | 0.229  | 0.130 |           |           |
|                    | III     | 7   | 0.392  | 0.0845|           |           |
| Salivary IgA (mg%) | I       | 3   | 19.46  | 8.30  | 6.45      | 0.000***  |
|                    | II      | 10  | 18.73  | 5.90  |           |           |
|                    | III     | 7   | 32.22  | 9.51  |           |           |
| Serum IgG (mg%)   | I       | 3   | 1243.66| 262.00| 5.109     | 0.018*    |
|                    | II      | 10  | 1679.70| 214.70|           |           |
|                    | III     | 7   | 1672.57| 201.61|           |           |
| Serum IgA (mg%)   | I       | 3   | 134.33 | 11.93 | 8.782     | 0.002***  |
|                    | II      | 10  | 220.10 | 43.73 |           |           |
|                    | III     | 7   | 255.42 | 46.00 |           |           |
| TSP g%            | I       | 3   | 6.03   | 0.90  | 7.103     | 0.006***  |
|                    | II      | 10  | 5.51   | 0.56  |           |           |
|                    | III     | 7   | 6.45   | 0.60  |           |           |
| Hb g%             | I       | 3   | 10.83  | 0.95  | 37.447    | 0.000***  |
|                    | II      | 10  | 11.13  | 0.64  |           |           |
|                    | III     | 7   | 8.01   | 0.82  |           |           |

*SS: Statistically significant; $P < 0.05$, **HVS: Highly statistically significant, $P < 0.01$, ***VHS: Very high statistically significant, $P < 0.001$. One-way ANOVA test; SD: Standard deviation, TSP: Total serum protein, Hb: Hemoglobin, Ig: Immunoglobulin

---

**Table 4: Pearson’s correlations of variables of oral submucous fibrosis**

| Parameters      | Salivary IgG (mg%) | Salivary IgA (mg%) | Serum IgG (mg%) | Serum IgA (mg%) | TSP (g%) | Hb (g%) |
|-----------------|--------------------|--------------------|-----------------|-----------------|----------|---------|
| Salivary IgG mg%| 1.000              | 0.578***           | -               | -               | -        | -       |
| Salivary IgA mg%| -                  | 1.000              | -               | -               | -        | -       |
| Serum IgG mg%   | $r=0.211$          | $r=0.272$          | 1.000           | -               | -        | -       |
| Serum IgA mg%   | $r=0.391$          | $r=0.246$          | $r=0.345$       | 1.000           | -        | -       |
| TSP g%          | $r=0.464*$         | $r=0.630***$       | $r=0.204$       | $r=0.617***$    | 1.000    | -       |
| Hb g%           | $r=-0.484*$        | $r=-0.630*$        | $r=-0.237*$     | $r=-0.487*$     | 1.000    | -       |

***Very high statistically significant; $P < 0.001$, *Statistically significant; $P < 0.05$. NS: Not significant, PC: Positive correlation, NC: Negative correlation, TSP: Total serum protein, Hb: Hemoglobin, Ig: Immunoglobulin
Conflicts of interest

There are no conflicts of interest.

References

1. Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Pindborg JJ, Mehta FS. Etiology of oral submucous fibrosis with special reference to the role of areca nut chewing. J Oral Pathol Med 1996;24:145-52.
2. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764-79.
3. Rajendran R. Oral submucous fibrosis: Etiology, pathogenesis, and future research. Bull World Health Organ 1994;72:985-96.
4. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: Review on etiology and pathogenesis. Oral Oncol 2006;42:561-8.
5. Dyavanagoudar SN. Oral sub mucous fibrosis: Review of etiopathogenesis. J Cancer Sci Ther 2009;1:72-7.
6. Yang SF, Hsieh YS, Tsai CH, Chou MY, Chang YC. The upregulation in vivo and in vitro in patients with oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:214-5.
7. Kerr AR. Efficacy of oral lycopene in the management of oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;103:214-5.
8. Murti PR, Gupta PC, Bhonsle RB, Daftary DK, Mehta FS, Pindborg JJ. Effect on the incidence of oral submucous fibrosis of intervention in the areca nut chewing habit. J Oral Pathol Med 1990;19:99-100.
9. Chaturvedi VN. Oral submucous fibrosis. Natl Med J India 1989;2:11-6.
10. Trivedy C, Meghji S, Warnakulasuriya KA, Johnson NW, Harris M, Copper stimulates human oral fibroblasts in vitro: A role in the pathogenesis of oral submucous fibrosis. J Oral Pathol Med 2001;30:465-70.
11. Ramachandran S, Rajeshwari GA, Sree GV. Pathogenesis of oral submucous fibrosis: The past and current concepts. Int J Oral Maxillofac Pathol 2012;3:27-36.
12. Prashanth G, Shantala R, Naik Sangamesh NC, Salivary IgA levels in patients with oral submucous fibrosis: A study. J Indian Acad Oral Med Radiol 2012;23:536-8.
13. Khanna JN, Andrade NN. Oral submucous fibrosis: A new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg 1995;24:433-9.
14. Cox SC, Walket DM. Oral submucous fibrosis. A review. Aust Dent J 1996;41:294-9.
15. Patidar KA, Parwani RN, Wanjari SP Correlation of salivary and serum IgG, IgA levels with total protein in oral submucous fibrosis. J Oral Sci 2001;1;53(1):97-102.
16. van Wyk CW, Grobler-Rabie AF, Martell RW, Hammond MG. HLA-antigens in oral submucous fibrosis. J Oral Pathol Med 1994;23:23-7.
17. Chiang CP, Shieh RP, Chen TH, Chang YF, Liu BY, Wang JT. High incidence of autoantibodies in Taiwanese patients with OSMF. J Oral Pathol Med 2002;31:402-9.
18. Pinakapani P, Shambulingappa P, Shashikanth MC. Salivary coagulopathy and immunoglobulins in Oral submucous fibrosis. J Indian Acad Oral Med Radiol 2009;21:62-6.
19. Tabak LA, Levine MJ, Mandel ID, Elison LA. Role of salivary mucins in protecting oral cavity. Journal of oral pathology and Medicine 1982;11:1-7.
20. Mandel ID. Salivary diagnosis: Promises, promises. Ann NY Acad Sci 1993;694:1-10.
21. Sirsat SM, Khanklar VR. Sub mucous fibrosis of the palate in diet. Pre conditioned Wister rats: Induction by local painting of capsacin-an optical and electron microscopic study. Arch Pathol 1960;70:171-9.
22. Sinor PN, Gupta PC, Murti PR. A case control study of oral submucous fibrosis with special reference to the etiologic role of areca nut. J Oral Pathol Med 1990;19:94-8.
23. Vanaja Reddy, Wanjari PV, Banda NR, Reddy P. Oral Submucous Fibrosis: Correlation of Clinical Grading to various habit factors. International journal of dental clinics 2011;3:231-35.
24. Van Wyk CW, Grobler-Rabie AF, Martell RW, Hammond MG. HLA antigens in oral submucous fibrosis. J Oral Pathol Med 1994;23:23-7.
25. Azz SR. Oral submucous fibrosis: An unusual disease. J N J Dent Assoc Spring 1997;68:17-9.
26. Aruna DS, R Prasad KV, Shavi GR, Ariga J, Rajesh G, Krishna M. Retrospective study on risk habits among oral cancer patients in Karnataka Cancer Therapy and Research Institute, Hubli, India. Asian Pacific. J Cancer Prev 2011;15:81-6.
27. Shah N, Kumar R, Shah MK. Immunological studies in Oral submucous fibrosis patients. Indian J Dent Res 1994;5:81-7.
28. Chaturvedi VN, Sharma AK, Chakrabarati S. Salivary coagulopathy and humoral response in oral submucous fibrosis. JIDA 1994;50:51-9.
29. Sato K. Enzyme linked immunosorbent assay of S-IgA in whole saliva of healthy subjects and patients with oral diseases. Bull Tokyo Med Dent Univ 1991;38:9-18.
30. Proctor GB, Carpenter GH. Chewing stimulates secretion of human salivary secretory immunoglobulin A. J Dent Res 2001;80:909-13.
31. DeRossi Scott S, Raghavendra S. Anemia. Oral Pathol Oral Med Oral Endo 2003; 95:131-41.
32. Taneja R, Jhangia B, Vaishali K. Hemoglobin levels in patients with oral submucous fibrosis. Oral Oncol 2006;42:561-8.
33. Lee YT. Quantitative change of serum protein and immunoglobulin in patients with solid cancers. J Surg Oncol 1977;9:179-87.