Original Article

Estimation of serum immunoglobulin G and immunoglobulin A levels in oral submucous fibrosis patients

ABSTRACT

Background: Oral submucous fibrosis (OSMF) is a disabling, chronic, insidious potentially malignant condition of the oral cavity seen predominantly in the Indian subcontinent. Due to the idiopathic nature and various immunological changes seen in some OSMF patients, it can be considered an autoimmune disorder. Hyperimmunoglobulinemia is often seen with OSMF.

Aim and Objectives: (i) To estimate the serum immunoglobulin G (IgG) and immunoglobulin A (IgA) levels in OSMF patients; (ii) to correlate the serum IgG and IgA levels with age and gender of OSMF patients; (iii) to correlate the serum IgG and IgA levels with clinical stages of OSMF; (iv) to correlate the serum IgG and IgA levels with the content of the habit in OSMF patients.

Materials and Methods: Serum samples were collected from a total of 50 patients (25 OSMF cases and 25 controls) of both genders and IgG and IgA levels were estimated by NEPHELOMETRY.

Results: As compared to the control group, the mean serum IgG level was marginally increased among the OSMF patients and the mean serum IgA level was marginally decreased among the OSMF patients, but this was not statistically significant. Furthermore, there was only a weak correlation between serum immunoglobulins and content of the habit and no significant correlation was found between the serum IgG and IgA levels and age and sex of the OSMF patients. Serum IgG was increased and IgA was decreased with increasing stages of OSMF.

Conclusion: The results of the present study suggested that there is an alteration in serum immunoglobulin levels in OSMF patients as compared to the control group.

Keywords: Immunoglobulin A, immunoglobulin G, immunoglobulins, oral submucous fibrosis

Anand N. Swami, Lata M. Kale[1], Sneha H. Choudhary[2], Ashok Kumar Singh[3], Sami D. Aldhuwayhi[4], Amar Thakare[4]

Swami Multispeciality Dental Clinic, Osmanabad, [1]Department of Oral Medicine and Radiology, C.S.M.S.S. Dental College and Hospital, Aurangabad, Maharashtra, [2]Department of Oral Medicine and Radiology, Alignment Dental Care, Kolkata, West Bengal, [3]Department of Oral Medicine and Radiology, Buddha Institute of Dental Sciences and Hospital, Patna, Bihar, India, [4]Department of Prosthodontics, College of Dentistry, Majmaah University, Al-Majmaah, Kingdom of Saudi Arabia

Address for correspondence: Dr. Sneha H. Choudhary, Consultant, Oral Medicine and Radiology, Alignment Dental Care, 2/5/2 Hindusthan Road, (1st Floor), Gariahat, Kolkata, West Bengal, India.
E-mail: snehachy1606@gmail.com

Received: 28 October 2020, **Revised:** 18 June 2021, **Accepted:** 12 October 2021, **Published:** 20 April 2022

INTRODUCTION

Oral submucous fibrosis (OSMF) was first described in 1952 by Schwartz who named it as "Atrophia idiopathica (tropicum) mucosum oris." Later, in 1953, it was first described in India.[1] OSMF, first defined in 1966 by Pindborg,[2] is a chronic debilitating disease of the oral cavity with insidious onset and associated involvement of pharynx and esophagus. Although there is worldwide distribution, OSMF is predominantly seen in the Indian population. The prevalence rate of OSMF in India which was once considered to be 0.2%–0.5% with a higher percentage being found in the southern part of the country, has now increased tremendously to 6.3% in the northern part of India.[3] The etiology of OSMF is multifactorial including

Access this article online	
Website: www.njms.in	Quick Response Code
DOI: 10.4103/njms.NJMS_238_20	

How to cite this article: Swami AN, Kale LM, Choudhary SH, Singh AK, Aldhuwayhi SD, Thakare A. Estimation of serum immunoglobulin G and immunoglobulin A levels in oral submucous fibrosis patients. Natl J Maxillofac Surg 2022;13:67-71.

local factors such as areca nut and betel quid chewing, ingestion of spicy red pepper and systemic factors, such as nutritional deficiency, hypersensitivity to various dietary constituents, and genetic and immunological susceptibility.[1] Furthermore, some characteristics of the disease suggest an autoimmune etiology such as the incidence of autoantibodies, the lymphocyte infiltration in the lesion, and the association with HLA-A10 and DR-3 genotypes.[4] To assess the status of humoral immunity, serum immunoglobulin levels are used as parameters. Though extensive studies have been done on etiopathogenesis, clinical presentation, and management of OSMF, only a few studies reveal correlation of serum immunoglobulins and clinical staging in such patients. Thus the present study was undertaken to highlight the role of humoral immune response in OSMF by estimation of serum immunoglobulin G (IgG) and immunoglobulin A (IgA) levels in different clinical stages of the disease.

MATERIALS AND METHODS

The present study comprised two groups (OSMF group and control group) of 25 patients each of either sex. The diagnosis of OSMF was made based on clinical features such as the presence of burning sensation in the mouth, intolerance to hot and spicy food, blanching of the oral mucosa, presence of fibrous bands, and restricted mouth opening, [Figure 1] which was further clinically divided into four grades as per the criteria described by Khanna JN and Andrade NN sin 1995.[5] Age- and sex-matched patients with no history of any adverse habits and no oral lesions constituted the control group. The OSMF group included patients in the age range of 18–60 years with the presence of clinical features of OSMF, and who agreed to the blood and immunological investigation. Patients having systemic diseases such as diabetes, hypertension, liver disease, chronic infection, coexistent lesions, or any immunological diseases were excluded from the study. The Ethical Committee clearance was obtained for the present study design from the Institutional Ethical Committee and after the receipt of the approval, the study was undertaken. The need and design of the study were explained to all the patients and only those who gave a signed informed consent were included in the study. The detailed case history was recorded for each patient.

Methodology

A volume of 2 ml blood sample was collected from each patient which was used for immunological investigation. Serum IgG and IgA were quantitated by using a diagnostic kit for NEPHELOMETRY: AGAPPE MISPA i2 [Figure 2] for estimation of immunoglobulin IgG and IgA by Nephlometry method. The reagents used for IgG are R1-Glycine Buffer Solution and R2-Latex suspension coated with denatured human IgG and for IgA are R1-Tris (hydroxysmethyl) aminomethane with PEG and R2-Anti-human IgA antiserum [Figure 3]. The test procedure and the calibration data as provided in the smart card along with the kit were accurately followed. The data thus collected were subjected to statistical analysis which included Student's *t*-test, Kolmogorov–Smirnov test and Shapiro–Wilk test, ANOVA test, and Spearman's rank correlation coefficient.

RESULTS

In the present study, out of 25 patients in the OSMF group, 19 were males and 06 were females. 4 male patients had grade I OSMF; 6 male and 2 female patients had grade II OSMF; 5 male and 3 female patients had grade III OSMF and 4 male and 1 female patient had grade IV OSMF. The mean age of the patient in the OSMF group was 32.20 years with a male: female ratio of 4:1. For correlating the serum IgG and IgA levels based on the age of the patients, they were divided into four age groups, namely., 19–30 years, 31–40 years, 41–50 years, and 51–60 years. Patients in the age group of

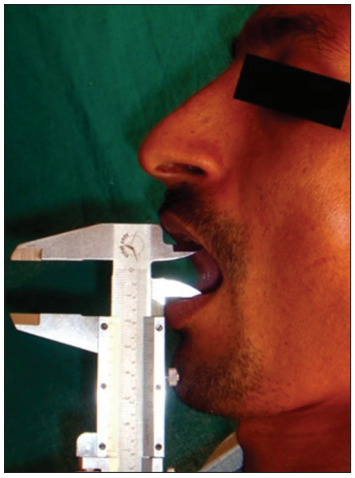

Figure 1: Measurement of interincisal opening using vernier caliper

Figure 2: Armamentarium for blood sample collection

19–30 years showed the lowest mean serum IgG levels which was gradually increasing to reach a maximum level in the age group of 51–60 years. The serum IgA levels showed no significant pattern of change and also the alterations were negligible [Table 1]. Statistically, Spearman's rank correlation of serum IgG and IgA levels with age groups of OSMF patients showed no correlation between age of the patient and serum IgG and IgA levels ($P > 0.05$).

The mean serum IgG levels in females were found to be more as compared to males. The mean serum IgA levels, in contrast, were found to be more in males as compared to females [Table 2]. However, statistically, the Bi-Serial Correlation of serum IgG and IgA levels with gender distribution showed only a weak correlation ($P > 0.05$). Also, the ANOVA test showed a gradual increase in IgG levels from the control group to grade IV OSMF cases, except for grade I which showed a marginal decrease in serum IgG levels as compared to control group. Serum IgA levels showed a gradual decrease in levels from control group to grade IV OSMF cases. However, these changes of serum IgG and IgA levels within the groups were statistically nonsignificant [Table 3].

Almost all female patients (except one) in the present study were habitual of areca nut chewing only whereas male patients had all types of tobacco-related habits including smoking. The mean serum IgG levels were found to be highest in patients with the habit of areca nut chewing alone and gradually decreased in patients having areca nut with gutkha, areca nut and gutkha with tobacco and those with areca nut and tobacco habit in smoke and smokeless form in the decreasing order. The mean serum IgA levels were highest among patients with areca nut alone habit and lowest in patients with areca nut and gutkha habit. Statistically, the Eta coefficient showed a weak correlation of habit patterns with serum IgG and IgA levels [Table 4].

Figure 3: NEPHLOMETER MEISPA i2 Diagnostic Kit And Machine

DISCUSSION

OSMF is a localized disease involving the oral cavity, pharynx, and upper part of the esophagus. Its clinical features range from burning sensation to complete fibrosis

Table 1: Correlation of serum immunoglobulin G and immunoglobulin A levels with age groups of oral submucous fibrosis patients

Age group	IgG		IgA	
	Mean±SD	*P*	Mean±SD	*P*
19-30	12.11±1.25	0.200	0.998±0.53	0.998
31-40	12.78±0.69		1.93±0.54	
41-50	12.87±0.42		1.85±0.68	
51-60	13.15±0.49		1.94±1.00	

SD: Standard deviation, IgG: Immunoglobulin G, IgA: Immunoglobulin A

Table 2: Correlation of serum immunoglobulin G and immunoglobulin A levels with sex of oral submucous fibrosis patients

	IgG			IgA		
	Mean±SD	Count	*P*	Mean±SD	Count	*P*
Male	12.47±0.92	19	0.101	1.93±0.58	19	0.947
Female	13.15±0.50	6		1.91±0.48	6	

SD: Standard deviation, IgG: Immunoglobulin G, IgA: Immunoglobulin A

Table 3: Comparison of all groups together

	n	Mean±SD	SE	Minimum	Maximum	*P*
IgG						
Control	25	12.0600±1.21792	0.24358	10.10	14.40	0.05
Group I	4	11.4500±1.20139	0.60069	10.50	13.90	
Group II	8	12.8875±0.62892	0.22236	11.80	13.60	
Group III	8	12.7375±0.73473	0.25976	11.80	13.60	
Group IV	5	13.0200±0.42661	0.19079	12.40	13.50	
Total	50	12.4080±1.09206	0.15444	10.10	14.40	
IgA						
Control	25	2.2700±0.68271	0.13654	1.24	3.69	0.286
Group I	4	2.1800±0.59257	0.29628	1.75	3.03	
Group II	8	2.0188±0.40180	0.14206	1.42	2.71	
Group III	8	1.8238±0.55749	0.19710	1.01	2.75	
Group IV	5	1.7420±0.75091	0.33582	1.12	2.65	
Total	50	2.0984±0.63652	0.09002	1.01	3.69	

SD: Standard deviation, IgG: Immunoglobulin G, IgA: Immunoglobulin A, SE: Standard error

Table 4: Correlation of serum immunoglobulin G and immunoglobulin A levels with habits of oral submucous fibrosis patients

	IgG			IgA		
	Mean±SD	Count	*P*	Mean±SD	Count	*P*
A	13.06±0.50	5	0.264	2.01±0.47	5	0.126
A + G	12.68±1.46	4		1.80±0.30	4	
A + G + T	12.54±0.91	11		1.95±0.63	11	
A + G + T + S	12.40±0.62	5		1.89±0.71	5	

SD: Standard deviation, IgG: Immunoglobulin G, IgA: Immunoglobulin A, A: Areca nut, G: Gutkha, T: Tobacco smokeless, S: Smoking tobacco

of the involved part of the oral cavity ultimately leading to trismus.[6] Several factors such as areca nut chewing, chili consumption, nutritional deficiency state, genetic susceptibility, autoimmunity, and collagen disorders have been suggested to be involved in the pathogenesis of this condition.[1] Based on clinical and epidemiological studies areca nut chewing is considered an important predisposing factor. However, the occurrence of OSMF in cases without any history of using irritants and with various immunological changes suggests that the disease is multifactorial, as is the case with oral cancer and most of its precursor lesions.[6]

In the present study, the age range of OSMF cases was found to be wide, i.e. 19–60 years, with maximum cases seen between 19 and 40 years, with the mean age being 32.20 years. Malempati *et al*.[7] have also found maximum cases between 20 and 40 years of age, with a mean age of 34.6 ± 12.42 years. Similar finding has been reported in previous studies with age ranging from 10 to 70 years.[8-10] Few studies have reported a higher number of occurrence of OSMF cases in the second and third decades of life which is in accordance with the present study.[8,9,11] On correlating the serum IgG and IgA levels with various age groups in OSMF patients, the serum IgG levels were found to be increasing gradually with the increase in age of the OSMF patients, whereas the serum IgA levels showed only trivial changes in different age groups. However, both these alterations were statistically insignificant. No other study has corelated the serum IgG and IgA levels with various age groups in OSMF patients.

In the present study, 76% were male and 24% were female suggesting an M:F ratio of approximately 4:1. Male predominance similar to the present study has been reported by various previous authors ranging from 2:1 to 34:1.[9-12] However, few studies have also reported female predominance in OSMF cases.[13,14] This gender predilection in the present study can be attributed to easy accessibility for male patients to areca nut and its products. The statistical correlation of serum IgG levels with gender distribution was found to be nonsignificant, but it showed a weak positive relation of the serum levels for females (13.2 ± 0.5 g/l) as compared to male patients (12.3 ± 0.9 g/l). In contrast to this, the serum IgA levels showed a negative relation for females (1.91 ± 0.48 g/l) as compared to male patients (1.93 ± 0.58 g/l). However, none of these findings were statistically significant. No other studies reported in the literature have correlated the serum IgG and IgA levels with gender.

In the present study, all OSMF cases had a habit of chewing areca nut in one or the other form. Grade I cases showed more habitual for areca nut with gutkha while Grade IV cases showed more liking for mixed habits including smoking. Furthermore, male patients were more inclined toward mixed habits whereas female patients had areca nut chewing habits only. It was clear from the obtained data that "areca nut" in any form is chief ingredient and the causative agent for OSMF. This has also been observed in previous studies.[15,16] Correlation of these habit patterns with serum IgG levels showed the levels to be highest in patients habitual to areca nut in single form. Furthermore, the levels were found to gradually decrease with the addition of tobacco products in both smokeless and smoke form. However, these changes in levels of serum IgG when compared statistically showed a weak correlation. Similarly, the serum IgA levels showed marginal decrease in levels from patients habitual to areca nut in single form to those with mixed habits. Again these changes were found to be statistically nonsignificant. These alterations in serum levels decreasing with the amount of mixture of areca nut and tobacco products can be attributed to less number of samples in each group. Furthermore, such correlation have never been reported previously in the literature, thus further studies are required to confirm such alteration in a larger sample size.

On the evaluation of serum IgG levels in control (12.06 ± 1.21 g/l) and OSMF cases (12.63 ± 0.87 g/l), we found the levels to be marginally increased in OSMF cases which was statically insignificant. This was in accordance with previous studies that have reported no change or statically insignificant rise in levels of serum IgG in OSMF cases.[7,17] However few studies have reported a statistically significant rise in levels of serum IgG in OSMF cases highlighting the role of the active immune phenomenon in osmf.[8-10,13,18] On further analyzing based on the OSMF grading we found an initial insignificant drop in IgG levels in grade I case followed by gradual increase from grade II to IV cases. A similar increase in levels of serum IgG in advanced stages of OSMF cases has been reported by Phatak[8] and Chaturvedi and Marathe.[9]

On the evaluation of serum IgA levels in control (2.27 ± 0.68 g/l) and OSMF cases (1.92 ± 0.54 g/l), we found the levels to gradually drop in OSMF cases which on statistics were found to be nonsignificant. This was in accordance with the previous studies that have reported statistically insignificant decrease in levels of serum IgA in OSMF cases.[7,9,10,13,17] However, few studies have reported a significant decrease in serum IgA levels in OSMF patients.[7,17] In contrast to our findings, few studies have reported rise in levels of serum IgA in OSMF cases which could be attributed to differences in the technique used for evaluation of serum immunoglobulin levels.[8,18-20]

CONCLUSION

The present study suggests that there are certain changes that occur in serum immunoglobulin levels which can be correlated with the disease progression in OSMF patients. Although it is difficult to say whether these changes are an active phenomenon or just secondary changes to the pathology. Considering the multifactorial etiology, this condition should be viewed from a broad perspective and a thorough evaluation of autoimmune phenomenon should be explored in larger sample size over a longer period to arrive at a definite conclusion.

Acknowledgments

We acknowledge each patient who participated in the present study for their co-operation throughout.

Declaration of patient consent

The authors declare that they have obtained consent from patients. Patients have given their consent for their images and other clinical information to be reported in the journal. Patients understand that their names will not be published and due efforts will be made to conceal their identity but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

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 1995;24:145-52.
2. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966;22:764-79.
3. Nigam NK, Aravinda K, Dhillon M, Gupta S, Reddy S, Srinivas Raju M. Prevalence of oral submucous fibrosis among habitual gutkha and areca nut chewers in Moradabad district. J Oral Biol Craniofac Res 2014;4:8-13.
4. Kamath VV, Satelur K, Komali Y. Biochemical markers in oral submucous fibrosis: A review and update. Dent Res J (Isfahan) 2013;10:576-84.
5. 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.
6. Pillai R., Balaram P, Reddiar KS. Pathogenesis of oral submucous fibrosis. Cancer 1992;69:2011-20.
7. Malempati S, Guttikonda VK, Vishnubhatla T, Neerupakam M, Koduri S, Buduru K. Role of immunological alterations in oral submucous fibrosis. J Indian Acad Oral Med Radiol 2019;31:24-8.
8. Phatak AG. Serum proteins and immunoglobulins in oral submucous fibrosis. Indian J Otolaryngology 1978;30:1-4.
9. Chatuvedi VN, Sharma AK, Chakrabarati S. Salivary coagulopathy and humoral response in oral submucous fibrosis (OSMF). J Indian Dent Assoc 1991;62:51-9.
10. Chaturvedi VN, Marathe NG. Serum globulins and immunoglobulins in oral submucous fibrosis. Indian Pract 1988;41:399-403.
11. Tupkari JV, Bhavthankar JD, Mandale MS. Oral submucous fibrosis (OSMF): A study of 101 cases. JIAOMR 2007;19:311-8.
12. Bose T, Balan A. Oral submucous fibrosis – A changing scenario. JIAOMR 2007;19:334-40.
13. Canniff JP, Harvey W, Harris M. Oral submucous fibrosis: Its pathogenesis and management. Br Dent J 1986;160:429-34.
14. van Wyk CW, Grobler-Rabie AF, Martell RW, Hammond MG. HLA-antigens in oral submucous fibrosis. J Oral Pathol Med 1994;23:23-7.
15. Lemmer J, Shear M. Oral submucous fibrosis – A possible case in a person of Caucasian Descent. Br Dent J 1967;82:646-54.
16. Shiau YY, Kwan HW. Submucous fibrosis in Taiwan. Oral Surg Oral Med Oral Pathol 1979;47:453-7.
17. Divya VC, Sathasivasubramanian S. Estimation of serum and salivary immunoglobulin G and immunoglobulin A in oral pre-cancer: A study in oral submucous fibrosis and oral lichen planus. J Nat Sci Biol Med 2014;5:90-4.
18. 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 Bioallied Sci 2016;8:S126-32.
19. Pinakapani R, Shambulingappa P, Shashikanth M. Salivary coagulopathy and immunoglobulins in oral submucous fibrosis. J Indian Acad Oral Med Radiol 2009;21:62-6.
20. Patidar KA, Parwani RN, Wanjari SP. Correlation of salivary and serum IgG, IgA levels with total protein in oral submucous fibrosis. J Oral Sci 2011;53:97-102.