

ORIGINAL ARTICLE

Estimation of Serum Immunoglobulins [IgG] and [IgA] in Patients with Grade III and Grade IV Oral Submucous Fibrosis

N.J.Mary¹, K Prashanth², A Sam Jaysheel³

1- Reader, Department of Biochemistry, Meghna Institute of Dental Sciences, Nizamabad.

2- Assistant Professor, Dept. of Biochemistry, Rajiv Gandhi Institute of Medical Sciences, RIMS Adilabad

3- PG Dept of Oral Medicine and Radiology, Meghna Institute of Dental Sciences, Nizamabad.

Abstract

Background: The present study was done to estimate the serum levels of IgG and IgA in patients of grade III and grade IV oral submucous fibrosis. **Materials & Methods:** A total of 189 patients of both sexes of OSMF of grade III and grade IV were selected for the study. Serum IgG and IgA were quantified by using a diagnostic kit (IgG and IgA turbidometric immunoassay for estimation of immunoglobulin IgG and IgA). **Results:** Maximum numbers of OSMF patients were found in the age group of 15 to 24 years (70.27%). Males were found to be more affected (78.30%) with OSMF than the female patients. On comparison of the OSMF and control patient's immunoglobulin values of IgG, it was found to be significant. Similarly the IgA levels were estimated and found to be statistically significant. **Conclusion:** Immunology has a definite role in OSMF and thus, there is imbalance of immunoregulation as well as alteration of local tissue architecture.

Keywords: Oral Submucous Fibrosis OSMF, Immunoglobulins, IgG, IgA

Address for correspondence: N.J. Mary, Reader Department of Biochemistry, Meghna Institute of Dental Sciences, Nizamabad Telangana State. Mobile: +919440293288 Email: mary_nokia@yahoo.co.in

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Introduction

Oral submucous fibrosis (OSMF) was first described by Schwartz JJ (1952) as "Atrophica idiopathica musosa oris" in Kenya followed by Joshi SG (1953) in India.¹ Higher rates of malignant transformation in OSMF have been reported. In this disease there is submucosal fibrosis affecting most of the oral cavity, pharynx and upper third of the esophagus leading to progressive trismus and dysphagia due to fibrosis.^{2,3} Oral submucous fibrosis is an insidious, chronic disease affecting any part of oral cavity and sometimes the pharynx. Occasionally it is preceded by and or associated with vesicle formation and is always associated with a juxtaepithelial inflammatory reaction followed by progressive hyalinization of the lamina propria.^{4,5} OSMF is indeed one of the classic "Disease of civilization" with large differences being seen between races, geographic areas and individuals at different

levels in both prevalence and the degree to which it transforms into malignancy with continuation of habit with increased frequency and duration.⁶ Serum immunoglobulin levels, which are used as biochemical markers to assess humoral immunity, continue to be an area of investigative research for their role in the pathophysiology of oral submucous fibrosis. The changes in serum immunoglobulin levels may appear much before the actual clinical symptoms. IgA is one of the main components of the adaptive immune system present in the saliva. Secretory IgA antibodies inhibit microbial adherence, metabolic pathways, colonization and penetration of the mucosal surfaces; neutralize enzymes, viruses, and toxins; mediate expulsion of plasmids and agglutination of microbes; and inhibit the growth of certain organisms. Plasma cells that are in close proximity to the secretory epithelial cells secrete IgA. The principal immunoglobulin in external secretions and in the entire body is IgA, and its role in protection against certain

mucosal pathogens is significant.^{7,8,9} The present study was done to estimate the serum levels of IgG and IgA in patients of grade III and grade IV oral submucous fibrosis.

Materials and Methods

A total of 189 patients of both sexes were involved in the study. The Study was carried out at Meghna Institute of Dental Sciences Nizamabad. Khanna JN, Dave R (1995) classification was used to classify the patients of OSMF into different grades and only patients of grade III and grade IV were selected for the study. Similar number of control group was also taken into the study. Permission from the Institutional Ethical Committee was obtained before starting the study. Informed consent was obtained from patients to participate in this study.

Inclusion criteria: OSMF patients of Group III and group IV

Exclusion criteria: Patients who were under medications such as immunomodulators or immunosuppressors, Patients with bleeding disorders and patients suffering from autoimmune diseases such as pemphigus, pemphigoid, systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, lichen planus, myasthenia gravis, Sjogren's syndrome, rheumatic fever, psoriasis, psoriatic arthritis, and chronic infections for both the groups. Physiological conditions such as pregnancy and menopause were also excluded.

Five milliliters of blood was collected by venipuncture using 24-gauge needles. From samples, blood serum was separated by centrifugation at 2500 rpm. Serum IgG and IgA were quantified by using a diagnostic kit (IgG

and IgA turbidometric immunoassay for estimation of immunoglobulin IgG and IgA). For estimation of serum IgG, 500 µl of IgG activation buffer R1 was taken in a clean cuvette. Serum sample was diluted in the ratio of 1:5 with normal saline. 5 µl of diluted serum sample was added to R1. After incubation of 10 min, 50 µl of R2 was added; i.e. 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. 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. Result was calculated accordingly.

Results

Patients of age 15 to 54 years were included in the study and divided according to the age group. Maximum numbers of OSMF patients were found in the age group of 15 to 24 years (70.27%). Males were found to be more affected (78.30%) with OSMF than the female patients.

Grade III and IV OSMF patients were taken for the study. On comparison of the OSMF and control patient's immunoglobulin values of IgG, it was found that highly significant difference. (Students t test, $P < 0.001$). Also the IgA levels were estimated and found statistically significant difference. (Students t test, $P < 0.001$) Table 2 shows the Number of Patients with Grade III and Grade IV Oral Submucosal Fibrosis along with sex distribution. Whereas there in general 148 (78.3%) were male and 41 (21.69%) were female. Most of the cases belong to Grade III OSMF 53.43% and 46.56 % were belonging to the Grade IV OSMF.

Table 1: Distribution of the patients according to the age and sex.

Age	Male		Female		Total n
	n	%	n	%	
15-24	104	70.27	11	26.82	115
25-34	25	16.89	6	14.63	31
35-44	11	7.43	6	14.63	17
45-54	8	5.4	23	56.09	31
Total	148	78.30	41	21.69	189

Table 2: Distribution of the patients according to Grades of OSMF.

Grade	n	%	Male	Female
Grade III OSMF	101	53.43	79	22
Grade IV OSMF	88	46.56	69	19
Total	189	100	148	41

Table 3: Levels of serum IgG & IgA in patients of OSMF according to different grades.

Group	Number of cases	IgG (Mean ± SD)	IgA (Mean ± SD)
OSMF	189	1786 ± 196.2	401 ± 87.3
Control	189	1121 ± 165.4	264 ± 57.3
T value		15.3421	13.4532
P value		<0.001*	<0.001*

*p<0.001 = statistically significant.

Discussion

The precancerous nature of OSMF has been emphasized by several authors and several Cases of OSMF has turned out to be Malignant. Since several factors have been associated with the etiology, there is a need to explore the etiology of OSMF.^{10,11} Most of the patients in our study were aged 15-35 years with the mean age being 24.30 years showing male predominance. These results were in agreement with previous studies, probably due to social encounter, economic liberty, popularity of refined areca nut products and easy availability of the product.³ Immunoglobulins are a heterogeneous 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.^{12,13} The role of active immune phenomenon in OSMF is supported by accelerated body defense 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. 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.^{3,12,13}

The prevalence of OSF has been found to be 0.41% in the present study. Pindborg et al reported prevalence of 0.04% in Andhra Pradesh to 0.4% in Kerala. Rajendran et al found prevalence to be 0.36% in Kerala, 0.04% in Andhra Pradesh and 0.16% in Gujarat in various house to house surveys.^{1,14} Nicotine significantly

reduces the secretory components, lactoferrin and lysozyme, by reducing the total cell numbers and their metabolic activity more in smokeless tobacco users than in nonusers. This could be the reason that smokeless tobacco users have decreased IgA levels. However, one possible reason could be that initially tobacco decreases, but consecutively increases the IgA levels in the saliva. Increased permeability leads to passive diffusion of IgG from the vascular and extravascular compartments into the saliva. This may be one of the reasons for the active inflammatory process. Increase in IgG and normal IgA levels are suggestive of only inflammation and no infection. Increase in secretory immunoglobulin A (S-IgA) in the affected individuals is suggestive of the reactive phenomena trying to limit the disease. S-IgA, which is one of the major surface defense mechanisms, increases in process of limiting the disease progression. Nicotine increases the mucosal permeability of the tissues, thereby increasing its penetration, and the mucosa tries to defend the surface by increasing the defensive mechanism. Secretory epithelial cells increase IgA secretion, thereby causing an increase in the diffusion of vascular IgA. Increase in immunoglobulin levels in oral submucous fibrosis patients signifies the role of an active immune phenomenon in the pathogenesis, with a possible autoimmune mechanism.^{9,10}

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. Abrol found results similar for salivary IgG but contrasting result for IgA. Phatak found normal levels of salivary immunoglobulins. 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 against the presence of disease. Kin KellaHeiner, 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.^{3,15,16,17}

Serum IgA levels in our study were raised significantly in Grade II ($P < 0.01$) and in Grade III ($P < 0.001$) cases compared to the control group. In contrast Chaturvedi and Marathe observed significant decrease in levels ($P < 0.01$) in Grade III cases as compared to the control group; Chaturvedi et al. reported decreased levels, but not significant ($P < 0.1$) in all the grades as compared to the control group. In the present study serum IgA level was significantly increased in Grade III compared to Grade I and Grade II cases ($P < 0.001$). In contrast Chaturvedi and Marathe observed IgA level significantly less in Grad III compared to Grade I and Grade II ($P < 0.001$) cases of OSMF.^{10,18,19}

Conclusion

Immunology has a definite role in OSMF and thus, there is imbalance of immunoregulation as well as alteration of local tissue architecture. 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.

Conflict of Interest: None declared

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Ethical Permission: Obtained

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