Original Article

Estimation of serum and salivary immunoglobulin G and immunoglobulin A in oral pre-cancer: A study in oral submucous fibrosis and oral lichen planus

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Abstract

Aim: Oral submucous fibrosis (OSMF) and oral lichen planus (OLP) are two frequently reported, potentially malignant disorders with multifactorial etiologies and ambiguous pathogenesis. An immunological pathogenesis has been hypothesized as a causative factor for both. The present study aims to evaluate the role of serum and salivary immunoglobulin G (IgG) and immunoglobulin A (IgA) in both these conditions, by their quantitative estimation. **Materials and Methods:** Saliva and serum samples were collected from 30 patients, clinically diagnosed and histopathologically confirmed with OSMF, 30 with OLP and 30 age and sex matched controls. The levels of IgG and IgA were estimated by nephelometry. **Results:** The mean values of serum IgG were marginally higher in both OSMF and OLP groups compared to the controls but this difference was not significant and the mean values of serum immunoglobulin A were marginally decreased in both the study groups compared to the controls but this difference was also not significant. Inconclusively low levels of salivary IgG and IgA were obtained in the three groups. **Conclusion:** The present study suggests an insignificant association of these immunoglobulins in the pathogenesis of both these diseases.

Key words: Immunoglobulin A, immunoglobulin G, nephelometry, oral lichen planus, oral submucous fibrosis

INTRODUCTION

The World Health Organization in 1978 categorized potentially malignant disorders of the oral cavity into two broad groups, as lesions and conditions.[1,2] A precancerous condition has the potential to undergo a malignant transformation, in any anatomical site of the mouth or pharynx. Oral submucous fibrosis (OSMF) and oral lichen planus (OLP) are two such frequently reported precancerous conditions.[3,4] Additionally, the various etiological factors implicated in these diseases, makes their treatment challenging.[4,5] An immunological pathogenesis has been hypothesized to be involved in both these conditions.[4-6] Therefore, this study was conducted to compare two precancerous conditions with different backgrounds and etiologies, where an immunological pathogenesis has been considered to play a role. Moreover, previous studies show a multitude of varying results. Our study wanted to analyze and confirm the outcome. Additionally, this study was to evaluate any significant relationship between the levels of immunoglobulins in serum and saliva.

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Immunoglobulins are glycoproteins expressed as membrane bound receptors on the surface of B cells or soluble molecules secreted from plasma cells.[7] Five distinct classes of immunoglobulin molecules namely IgG, IgA, IgM, IgD and IgE are recognized in humans. IgG is the predominant immunoglobulin in normal serum (70-75%, approximately 1000 mg/dl).[8] IgA is the next most predominant immunoglobulin, accounting for approximately 15-20% (approximately 200 mg/dl).[7] Secretory IgA constitutes the predominant immunoglobulin

isotype in secretions, including saliva.[9] In saliva, the IgG level is estimated to be approximately 2-3 mg/dl as compared with the IgA concentration in saliva which is around 10-20 mg/dl.[10] Literature review reveals multifarious observations, with increased, decreased and even normal levels of IgG and IgA. Hence this study was designed to quantitatively evaluate IgG and IgA in serum and saliva of OSMF and OLP, thereby to observe any possible association of these immunoglobulins in the pathogenesis of these diseases and additionally to assess the value of saliva as a reliable substitute.

MATERIALS AND METHODS

The study (self-financed) was conducted at Sri Ramachandra University (Chennai, India), following approval from the Institutional Ethical Committee Majority of the subjects for the study were selected from the patients reporting to the Department of Oral Medicine and Radiology and some patients from the Department of Dermatology. All the patients who participated in the study were explained about the same and an informed consent was taken. They were grouped based on the criteria as stated below. The study group included a total of 90 individuals who were divided into three groups namely, "Group 1" comprised of controls, "Group 2" comprised of OSMF and "Group 3" comprised of OLP. The OSMF and OLP cases were identified on the basis of their clinical features and subjected to biopsy procedures for the histopathological confirmation.

Individuals with clinical features of OSMF and associated habits of betel/paan chewing were included.[11] Individuals with clinical manifestations of OLP, based on the WHO diagnostic criteria, were included.[12] Individuals suffering from any immunologically associated diseases and those with an immunocompromised state were excluded from the study.[13,14] Those cases exhibiting lesions similar to OLP but with associated habit of betel/tobacco chewing were excluded, in order to avoid including cases with lichenoid reaction.[4,14] Cases with prior history of treatment for these disease were also excluded.[13]

Age and sex matched controls were selected from the same population as the study subjects. Individuals with any immunologically associated diseases or immunocompromised states or currently on any medications were excluded from the study. Three milli liters of blood was collected by venipuncture and centrifuged. Following which the serum that was separated, was transferred into storage vials. Two milli liters of unstimulated whole saliva, was collected by spitting method, in sterile tubes and centrifuged. Following which the clear fluid was drawn out with the help of disposable plastic pipettes, carefully excluding the sediment at the bottom, and transferred into storage vials.

A total of 180 samples (90 samples of serum and 90 samples of saliva) were estimated for the IgG and IgA levels by a Dade Behring BN ProSpec Nephelometer (at Sri Ramachandra University). The reagents (N Antiserum to Human IgA; N Antiserum to Human IgG; N/T Protein Control SL/M; N Protein Standard SL) and diluents (N Diluent; N Reaction Buffer) were obtained from Siemens, Chennai, Tamil Nadu, India.

RESULTS

In the present study the reference range for the serum samples was 7.0-16.0 g/L for IgG and 0.7-4.0 g/L for IgA. The data were subjected to statistical analysis and the statistics used is the Student independent *t*-test for comparison of two groups and the three groups were compared with each other by Oneway ANOVA. With the exception of a very few values, the salivary values were shown mostly as <0.0683 g/L in case of IgG and < 0.231 g/L for IgA. Since the values were not a definite one, it could not be statistically analysed.

DISCUSSION

OSMF and OLP are two widely encountered, potentially malignant disorders, amongst the Indian population. Multiple etiology and pathogenesis have been suggested for these disorders, including an immunological basis, involving factors such as immunoglobulins,[15,16] Beta2 micro-globulins,[17,18] autoantibodies[13,19-21] amongst others. Various prior immunological studies have reported wide ranging observations, with increased,[15,16,20,22,23] decreased[21,23-25] and even normal levels of immunoglobulins.[19,26-31] Among the five distinct classes of immunoglobulins, IgG and IgA are the most abundant immunoglobulins with an availability of 75% and 15% in the serum.[7] Moreover, literature reveals that IgG and IgA are the widely studied immunoglobulins in these disorders.[15,16,18,19-41] Hence, substantiating the choice of estimating the levels of IgG and IgA in OSMF and OLP.

The present study included three groups, individually comprising of 30 patients with OSMF and OLP, and a third group comprising of 30 age and sex matched controls. Previously treated individuals and those with other immunologically associated diseases or immunocompromised states were excluded to avoid related chances that may mask the changes in the immunoglobulin levels. The presence of a control group was indispensable

as, no prior set standards are available, any variations or on the basis of which an individual can be assessed.

Most of the previous studies have used the technique of radial immunodiffusion (RID) for assessing immunoglobulins.[15,20,23-25,27,28,30,31] Though the method is simple to perform and requires very little equipment, it has limitations including relative imprecision of the assay, dependence on antigen quantity and configuration and the amount of time consumption.[42] According to recent inter-laboratory proficiency surveys from the College of American Pathologists, the vast majority of clinical laboratories use nephelometric methods to measure immunoglobulins.[42] Fewer than 2% use RID. Hence, the present study quantitatively estimated the immunoglobulins by nephelometry, using a Dade Behring BN Prospec nephelometer.

Since whole saliva can be collected non-invasively, and by individuals with limited training and reflects systemic changes in several instances, it was included to observe the presence of any significant correlation between immunoglobulin levels in serum and saliva.[43] Though several studies previously have estimated levels in serum, very few have made any observations in saliva, of individuals with OSMF and OLP.[22,39,40]

The results of the present study clearly show that the mean values of serum IgG were only marginally higher (IgG = 13.65 g/L) [Table 1] and serum IgA were negligibly decreased in OSMF (IgA = 2.42 g/L) [Table 2] when compared to the control group (IgG = 13.12 g/L; IgA = 2.47 g/L) but this difference was not statistically significant. These results are in accordance with prior studies in which the serum IgG and IgA levels in the OSMF did not show any significant statistical difference when compared to controls.[18,21,31,32]

Similarly, on comparing the mean value of serum IgG in OLP (IgG = 13.57 g/L) groups to the controls (IgG = 13.12 g/L), it showed only a marginal increase [Table 3]. The serum IgA levels in OLP (IgA = 2.29 g/L) [Table 4] was marginally decreased when compared to that of the controls (IgA = 2.47 g/L). But these differences were not statistically significant. This is in accordance with previous studies in which the both the serum IgG and IgA of lichen planus cases did not show any significant statistical difference compared to the controls.[25,29-33,38] However, in a study by Lundstrom 1985, only the serum IgA did not show any significant variation compared to the controls.[21] Likewise, studies by Rajiv Gupta in 1994 and Dolores Bioina– Lukenda in 2008 showed no significant differences in the levels of only serum IgG in case of lichen planus compared to the control group.[35,41]

Table 1: Comparison of serum immunoglobulin G (oral submucous fibrosis versus control)

Groups	No. of patients	Mean±SD (g/L)	Student's independent *t*-test
2 OSMF	30	13.65±2.69	*t*=0.87; *P*=0.38
1 control	30	13.12±1.91	DF=58, not significant

*Significant at *P*≤0.05 **Highly significant at P≤0.01, ***Very high significant at *P*≤0.001, The mean value of IgG in serum of group 2 is higher than that of group 1 and subsequent to the *t*-test the *P* value was 0.38 which is not statistically significant, OSMF: Oral submucous fibrosis, DF: Degrees of freedom

Table 2: Comparison of serum immunoglobulin A (oral submucous fibrosis versus control)

Groups	No. of patients	Mean±SD (g/L)	Student's independent *t*-test
2 OSMF	30	2.42±1.00	*t*=0.19; *P*=0.85
1 control	30	2.47±0.86	DF=58, not significant

*Significant at *P*≤0.05 **Highly significant at *P*≤0.01 ***Very high significant at *P*≤0.001, The mean value of IgA in serum of group 2 is lesser than that of group 1 but it is not statistically significant, with a *P* value of 0.85, OSMF: Oral submucous fibrosis, DF: Degrees of freedom

Table 3: Comparison of serum immunoglobulin G (oral lichen planus versus control)

Groups	No. of patients	Mean±SD (g/L)	Student's independent *t*-test
3 OLP	30	13.57±1.87	*t*=0.92; *P*=0.36
1 control	30	13.12±1.91	DF=58, not significant

*Significant at *P*≤0.05 **Highly significant at *P*≤0.01 ***Very high significant at *P*≤0.001, The mean value of IgG in serum of group 3 is higher than that of group 1 and subsequent to *t*-test the *P* value was 0.36 which is not statistically significant, OLP: Oral lichen planus, DF: Degrees of freedom

Table 4: Comparison of serum immunoglobulin A (oral lichen planus versus control)

Groups	No. of patients	Mean±SD (g/L)	Student's independent *t*-test
3 OLP	30	2.29±1.05	*t*=0.71; *P*=0.47
1 control	30	2.47±0.86	DF=58, not significant

*Significant at *P*≤0.05 **Highly significant at *P*≤0.01 ***Very high significant at *P*≤0.001, The mean value of IgA in serum of group 3 is lesser than that of group 1 but it is not statistically significant, with a *P* value of 0.47. OLP: Oral lichen planus, DF: Degrees of freedom

Though various reasons were attributed to the insignificant differences in the levels between patients and controls, according to Rodriguez *et al.* (2001), patients with lichen planus did not show systemic immunological alterations and the humoral immune system alterations observed previously might in fact be the result of damage to basal keratinocytes.[38] Similarly, Scully (1982) supported the evidence of cell mediated immunopathogenesis for lichen planus, particularly since the mononuclear cell infiltrate beneath the epithelium is mainly T lymphocytes.[31] These reasons justify the results of our study.

According to Haque (1997) there were only occasional scattered B lymphocytes in the mononuclear cell infiltrates

in OSMF specimens, and in some lesions they were not found.[44] This is similar to the findings in oral cancers and precancers and suggests that humoral immunity plays only a minor role. Moreover the high ratio of CD4 to CD8 in OSMF tissues suggests an ongoing cellular immune response leading to possible imbalance of immunoregulation and alteration in local tissue architecture. Chiang *et al.*(2002) showed that significant increase in the number of T lymphocytes and macrophages and a predominance of CD4 lymphocytes over CD8 lymphocytes in the subepithelial connective tissue of OSMF specimens.[13] Macrophages and B lymphocytes are the minor immunocompetent cells in the subepithelial connective tissue and are only occasionally found in the epithelium of OSMF specimens. It was implied that cellular immune response may play an important role in the pathogenesis of OSMF. This could be the reason for the insignificant differences of the values of serum IgG and IgA between OSMF and controls, in our study also.

The levels of IgG and IgA obtained in the saliva samples were determined only in very few samples and could not be detected precisely in most of the samples. This could be attributed to the sensitivity of the equipment to precisely measure the levels of IgG and IgA or the non-availability of a modified nephelometric method like Particle Enhanced Nephelometric Immunoassay, which has been used for evaluation of saliva.[45] The present study showed no significant differences in the serum IgG and IgA values of OSMF, OLP and control groups. Hence an association of IgG and IgA in both these conditions could not be definitely ascertained.

CONCLUSION

The results of the present study suggests that quantification of IgG and IgA does not show any significant increase or decrease in these two diseases [Table 5] suggesting an insignificant association of these immunoglobulins in the pathogenesis of both these diseases. An association of a cellular immune response in these diseases, needs to be probed.

Table 5: Comparison of serum IgG and IgA between control, oral submucous fibrosis and oral lichen planus

Groups	*N*	Mean (g/L)	SD	Oneway ANOVA
Serum IgG (g/l)				
Control	30	13.1163	1.91378	*F*=0.51
OSMF	30	13.6460	2.69265	*P*=0.60
OLP	30	13.5667	1.8609	Not significant
Serum IgA (g/l)				
Control	30	2.473000	0.8617830	*F*=0.26
OSMF	30	2.427333	1.0021870	*P*=0.76
OLP	30	2.295133	1.0534107	Not significant

Oneway ANOVA was used to compare the serum IgG and IgA values between the 3 groups, No significance between the serum IgG and IgA values amongst the 3 groups, The above analyses suggests that there is no significant differences in the serum IgG and IgA levels between the 3 groups, OSMF: Oral submucous fibrosis, OLP: Oral lichen planus, IgA: Immunoglobulin A, IgG: Immunoglobulin G, SD: Standard deivation

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