J Med Sci 2017;37(5):195-200
DOI: 10.4103/jmedsci.jmedsci_29_17

ORIGINAL ARTICLE

Immunoglobulin A in Oral Potentially Malignant Disorders and Oral Squamous Cell Carcinoma

Vidya Ajila[1], Veena Shetty[2], Subhas Babu[1], Shruthi Hegde[1], Shama Rao[3]

[1]Department of Oral Medicine and Radiology, A B Shetty Memorial Institute of Dental Sciences, Nitte University, [2]Department of Microbiology, K S Hegde Medical Academy, Nitte University, [3]Stem Cell Research and Regenerative Medicine, Nitte University Centre, K S Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India

Introduction: Oral squamous cell carcinomas (OSCCs) are malignancies which develop predominantly from preexisting potentially malignant disorders. Immunoglobulin A (IgA) in saliva and serum is responsible for local immune response. The present study evaluates the role of serum and salivary IgA in OSCC and oral potentially malignant disorders (OPMDs). **Materials and Methods:** Ninety individuals were recruited into the study in three groups of thirty each. The participants were categorized as control group, study Group I consisting of OPMDs, and study Group II consisting of OSCC. Serum and salivary IgA was analyzed using nephelometry. **Results:** Serum IgA showed statistically significant increase between controls and study Group II and between study Group I and study Group II ($P < 0.05$). Salivary IgA showed statistically significant difference between controls and study Group I and between controls and study Group II ($P < 0.05$). **Conclusion:** The results show increased serum IgA in OSCC group as compared to controls while salivary IgA increased in both OPMD and OSCC groups. Thus, the present study indicates a role for serum IgA in OSCC while salivary IgA has potential as a prognostic indicator in OPMDs and OSCCs.

Key words: Immunoglobulin A, serum, saliva, oral potentially malignant disorders, oral squamous cell carcinoma

INTRODUCTION

Malignancy of the oral cavity is the sixth most prevalent cancer in the world most commonly being diagnosed as oral squamous cell carcinoma (OSCC).[1] Oral cancer ranks third among cancers in South and Central Asia.[2] In India, it is the most common cancer in males and the third most common cancer in females.[3] OSCC makes up 95%–98% of all oral cancers.[4] Despite the advances in oncology and surgery, the mortality rate of OSCC remains undiminished.[2] Thus, OSCC constitutes a major health challenge with increased need for research and identification of biomarkers which can help predict malignant transformation.

Oral potentially malignant disorders (OPMDs) were earlier described as "precancerous lesions and precancerous conditions." However, since all lesions do not progress to malignancy but only possess the potential for malignant transformation, they are now clubbed together as "OPMDs."[5] A study from India reported an annual malignant transformation rate of 0.3% while Western countries report an annual malignant transformation rate of upto 1%.[6]

The major etiology of OSCC is the habit of tobacco chewing and smoking. In South-East Asian countries like India, another etiological factor is the use of areca nut leading to the occurrence of oral submucous fibrosis (OSMF) which is an OPMD.[1] Other OPMDs include leukoplakia, erythroplakia, erosive lichen planus, discoid lupus erythematosus, and palatal lesions due to reverse smoking.[7] Most OSCCs are preceded by OPMDs.[8]

Prevention and early detection of OPMD helps in the avoidance of oral cancer and the associated mortality and morbidity. Therefore, there is a need to identify markers that can indicate the occurrence of OPMD and OSCC.

Salivary immunoglobulins (Igs) are implicated in the local immune response of the oral mucosa because irritants remain

Received: March 03, 2017; Revised: May 23, 2017; Accepted: June 30, 2017

Corresponding Author: Dr. Vidya Ajila, Department of Oral Medicine and Radiology, A B Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore, Karnataka, India. Tel: +918242204572; Fax: +918242204776. E-mail: ajila_v@yahoo.com

How to cite this article: Ajila V, Shetty V, Babu S, Hegde S, Rao S. Immunoglobulin A in oral potentially malignant disorders and oral squamous cell carcinoma. J Med Sci 2017;37:195-200.

longer in the saliva which constantly bathes the oral mucosa.[9] Saliva is an attractive alternative medium as compared to serum since it can be obtained noninvasively and in all populations.[9]

IgA is the second most common Ig in serum and found extensively in secretions such as saliva, mucous, colostrum, and tears. It is, therefore, implicated in humoral immunity.[10] Studies have tried to determine the utility of salivary IgA as a biomarker in oral precancer and cancer. Researchers have found increased levels of serum IgA in patients with OSCC as compared to healthy controls.[11] It has also been reported that the levels of serum Igs increase with the progression of the disease.[11] Since IgA is associated with local immune response and saliva is in direct constant contact with oral lesions, salivary IgA is proposed to accurately reflect the changes caused by OPMD and OSCC in the oral cavity. The increase in salivary IgA in OSCC is proposed to be due to increased local infection, increased antigenic inflammatory stimulus, increased local synthesis, and local host reaction to the disease.[9]

The present study is thus aimed to determine the usefulness of serum and salivary IgA as a diagnostic and prognostic marker in OPMD and OSCC.

MATERIALS AND METHODS

A total of ninety individuals reporting to the outpatient department of our dental hospital were included in the study. They were categorized into three groups of thirty individuals each. Study Group I included individuals diagnosed with OPMDs such as leukoplakia, OSMF, and erosive lichen planus. Study Group II included patients diagnosed with OSCC and the third group comprised healthy controls. Individuals who were already under treatment for the above conditions were excluded from the study.

Institutional ethical clearance was obtained for the study. After obtaining informed consent and performing oral examination, saliva and serum were collected. Saliva was obtained using spit method and stored at −20°C until analysis. Venous blood was obtained and serum was extracted. Serum was then stored at −20°C until further analysis. Serum and salivary IgA was analyzed using nephelometry (Agappe Diagnostics, Kerala, India). Data obtained were then subjected to statistical analysis.

Statistical analysis

The three groups were compared using Kruskal–Wallis test. Mann–Whitney U-test was used for intergroup comparison. Chi-square test was used to compare the gender differences and periodontal status between the groups. ANOVA was used to compare the age differences and the decayed, missing, and filled teeth (DMFT) status of the three groups.

RESULTS

Both study groups showed male predominance with study Group I having 86% of males and study Group II having 76% of males. A majority of OPMD cases were in the fifth decade, while in OSCC, the maximum cases were in the sixth decade. Comparison of the baseline characteristics such as age and gender showed statistically significant difference between the groups [Tables 1 and 2]. The most common potentially malignant disorder was OSMF [Table 3]. Gutkha chewing and smoking were the habits associated with OSMF and leukoplakia, respectively. In the OSCC group, the buccal mucosa was the most common site and betel quid chewing was the most common etiological factor. The details regarding location and staging of OSCC are given in Tables 4 and 5. Comparison of periodontal status and DMFT values also showed statistically significant difference [Tables 6 and 7].

Serum immunoglobulin A

The mean level of IgA in the control, OPMD, and OSCC groups was 157.51 (standard deviation [SD] ±72.16), 157.40 (SD ± 102.74), and 212.62 (SD ± 100.56) mg/dl, respectively. On comparison, there was statistically significant difference between control group and study Group II ($P = 0.005$) and also between study Group I and study Group II ($P = 0.004$). However, the difference between the control group and study Group I was nonsignificant ($P = 0.71$) [Figure 1]. $P < 0.05$ was considered statistically significant.

Table 1: Age comparison

	N	Mean±SD	Minimum	Maximum	ANOVA	
					F	*P*
1 (I)	30	43.60±12.14	23	65	49.06	<0.001*
2 (II)	30	56.37±8.43	42	73		
3 (C)	30	30.41±9.20	15	47		

*P<0.05 statistically significant, P>0.05 NS. NS=Nonsignificant; SD=Standard deviation

Table 2: Gender comparison between the groups

Gender	Group I (%)	Group II (%)	Group III (%)	Total (%)
Female	4 (13.3)	7 (23.3)	16 (53.3)	27 (30.0)
Male	26 (86.7)	23 (76.7)	14 (46.7)	63 (70.0)
Total	30	30	30	90
		χ^2 (2)=12.38, P<0.001*		

*P<0.05 statistically significant, P>0.05 NS. NS=Nonsignificant

Table 3: Distribution of oral potentially malignant disorders

Lesion	OSMF			Leukoplakia		Erosive lichen planus
Number of patients	19			10		1
	Stage 1	Stage 2	Stage 3	Homogeneous	Speckled	
	1	11	7	8	2	

OSMF=Oral submucous fibrosis

Table 4: Location of oral squamous cell carcinomas

Site	Buccal vestibule	Alveolo-gingival sulcus	Hard palate	Alveolus	Buccal mucosa	Labial mucosa	Tongue	Floor of mouth	Soft palate	Buccal mucosa + hard palate	Alveolus + hard palate	Alveolar mucosa + tongue
Number of patients	2	3	2	2	9	3	4	1	1	1	1	1

Table 5: Staging of oral squamous cell carcinomas

Stage	Stage 1	Stage 2	Stage 3	Stage 4
Number of patients	5	5	6	13

Table 6: Comparison of periodontal status between the groups

	Group I (%)	Group II (%)	Group III (%)	Total (%)
Periodontal status				
Gingivitis	12 (40.0)	3 (10.0)	6 (20.0)	21 (23.3)
Healthy gingiva	2 (6.7)	0	22 (73.3)	24 (26.7)
Periodontitis	16 (53.3)	27 (90.0)	2 (6.7)	45 (50.0)
Total				
Count	30	30	30	90
		χ^2 (4)=63.93, P<0.001*		

*P<0.05 statistically significant, P>0.05 NS. NS=Nonsignificant

Table 7: Comparison of decayed, missing, and filled teeth values between the groups

	n	Mean±SD	Minimum	Maximum	ANOVA	
					F	*P*
1	30	2.93±1.617	0	6	9.09	<0.001*
2	30	4.63±1.712	2	8		
3	30	3.23±1.612	0	6		

*P<0.05 statistically significant. SD=Standard deviation

Salivary immunoglobulin A

The mean level of IgA in the control, OPMD, and OSCC groups was 75.51 (SD ± 8.13), 89.53 (SD ± 54.10), and 94.47 (SD ± 26.90) mg/dl, respectively. On comparison, there was statistically significant difference between control and study Group I (P = 0.04) and also between control group and study Group II (P = 0.006). However, study Group I and study Group II had no significant difference (P = 0.66) [Figure 2]. P < 0.05 was considered statistically significant.

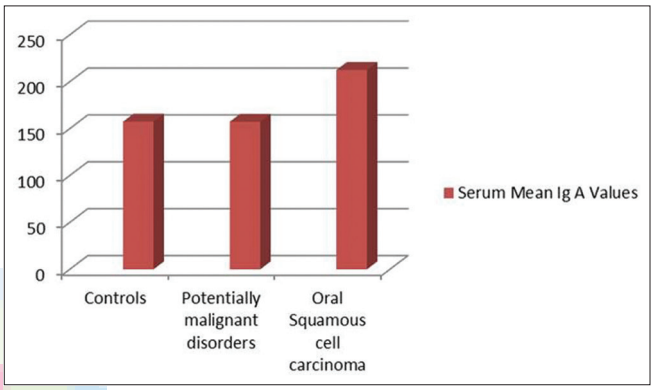

Figure 1: Increased mean levels of serum immunoglobulin A in oral squamous cell carcinoma patients as compared to controls. Serum immunoglobulin A was similar in controls and oral potentially malignant disorder groups. Statistically significant difference (P < 0.05) was present between controls and oral squamous cell carcinoma groups and between oral potentially malignant disorder and oral squamous cell carcinoma groups

DISCUSSION

OSCC is a significant health problem in India with high mortality and morbidity. Prevention of oral cancer development is the keystone to management.

The present study evaluated the serum and salivary IgA in OSCC and OPMD patients as compared to healthy controls. More prevalence of OPMD and OSCC was found in males. This is attributed to the increased incidence of substance abuse in males.[4,7] However, Neville and Day.[12] stated that the difference in male:female ratio has changed in the recent years due to increased exposure of women to carcinogenic habits such as smoking and alcohol consumption. The present study showed a male:female ratio of 6:1 and 3:1 in OPMD and OSCC patients, respectively.

OPMD occurred earlier in life as compared to OSCC. The early incidence of OPMD may be due to easy access, peer pressure, or media advertisements of tobacco products.[7] The later age of onset of OSCC in our study could be due to increased exposure period to risk factors and also due to

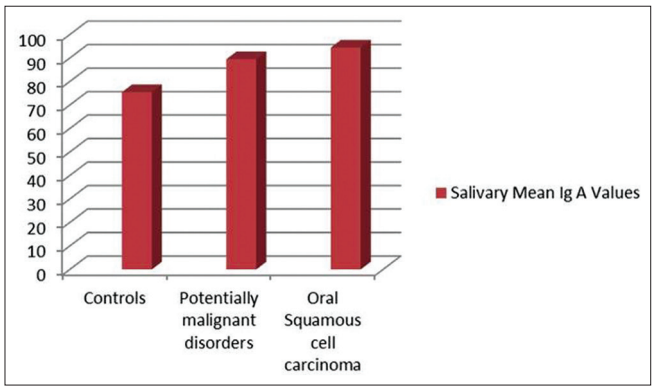

Figure 2: Increased mean levels of salivary immunoglobulin A in oral potentially malignant disorder and oral squamous cell carcinoma patients as compared to controls. Statistically significant difference ($P < 0.05$) was present between control and oral potentially malignant disorder groups and between control and oral squamous cell carcinoma groups

age-related immunological factors.[1] The highest incidence of OPMD in the present study was in the fifth decade and of OSCC was in the sixth decade. This difference could also be related to the time needed for conversion of OPMD to OSCC. The present study showed that the difference in the age of patients in OPMD group when compared to those of OSCC group was statistically significant.

The most common site for OSCC was the buccal mucosa. In India, 60% of oral cancers affect the buccal mucosa, lower alveolus, and the retromolar trigone together termed as "cancer of gingivo-buccal complex."[13] In Western countries, the tongue and the floor of the mouth are commonly affected.[1]

Tobacco smoking and chewing are implicated strongly in the etiology of OPMD such as leukoplakia, OSMF, and erythroplakia. India is the second largest producer of tobacco in the world.[14] Beedi and cigarette, paan, gutkha, khaini, etc., are the various forms of tobacco widely used in India.[14] The risk ratio for OSCC was estimated as four fold in chewers, two fold in smokers, and four fold in chewer-smokers.[13] In addition, smoking and paan chewing have been associated with poorer prognosis in patients diagnosed with OSCC.[15] Most patients with leukoplakia, OSMF, and OSCC had the habit of tobacco usage in the present study. OSMF was associated with areca nut chewing either in the form of quid (21%) or as gutkha (68%) which is processed areca nut.

Igs are glycoproteins produced from B-cells and plasma cells. IgA constitutes the predominant Ig in secretions such as saliva and is the second largest Ig in the serum. Various studies have investigated the role of IgA in both potentially malignant disorders and in oral cancer with conflicting results.[16]

Brown *et al*.[17] tried to correlate serum and salivary IgA to progression of disease in OSCC. They found elevated levels of serum and salivary IgA in OSCC patients with a possible role of IgA in detecting recurrent disease. Shilpashree and Sarapur[18] have mentioned that smoking, which is a major causative factor for OPMD and OSCC, can alter the Ig profile of the saliva. Phillips *et al*.[19] correlated the levels of salivary IgA with mortality in the general population. They found that low levels of IgA in the saliva were associated with increased risk of mortality from cancer other than lung cancer. They also state that, in oral diseases, low IgA levels are seen in dental caries, and high IgA levels are a marker of current oral infection. Parveen *et al*.[11] evaluated serum Igs in patients with different American Joint Committee on Cancer staging and found significant elevation of serum IgG, IgA, and IgM in all OSCC patients. Increase in IgA levels was noted with the progression of the disease with higher levels in advanced disease as compared to early disease. In the present study, IgA levels were increased in OSCC patients when compared to controls. Thus, our results are in accordance with previous reports which state an increase in IgA in serum and saliva in OSCC cases.

The elevated IgA blood levels could reflect the autoimmune nature of cancer and may be of use in the detection of recurrent disease. The increase in salivary IgA could be in response to the local changes in the mucosa.

Taneja *et al*.[20] evaluated the role of serum Igs in patients with OSMF. They found no significant change in IgA levels but increased serum IgG and IgM. This is similar to the present study where no significant change was observed in serum IgA between control group and OPMD group. Significant increase of serum IgA was seen in OSCC cases, thus suggesting a role for serum IgA in OSCC.

Krasteva *et al*.[21] found significantly higher IgA in saliva of patients with OSCC. The present study showed significant increase between control and OPMD groups and also between control and OSCC groups. Thus, salivary IgA levels increased progressively from controls to OPMD to OSCC even though the difference between the groups was not significant. Thus, salivary IgA level is a potential prognostic indicator in OPMD and OSCC.

However, there are limitations to our study. Since IgA is responsible for local host immune response, various other factors can cause increased IgA levels. These factors include age and gender of the individuals, smoking and alcohol habits, and periodontal status. Jafarzadeh *et al*.[22] found that salivary IgA levels increase with age till 60 years and then gradually decrease. Gonzalez-Quintela *et al*.[23] demonstrated that serum IgA levels increase with age and it is more common in males as compared to females. Whereas few authors report higher levels in females as compared to males and have correlated these findings to either hormonal variations or decreased salivary flow rate.[24] Salivary IgA levels also increase in patients with

periodontal disease.[25] Although alcohol can cause increase in IgA, smoking decreases IgA levels but it is associated with increased periodontal disease.[26] Higher IgA levels have been associated with decreased incidence of caries.[22] Since increased age, tobacco habits such as smoking, and periodontal disease are found in individuals with OPMD and OSCC, we could not completely eliminate these confounding factors from the present study.

CONCLUSION

Igs such as IgA mediate the local immune response of the oral cavity and could be an indicator of developing OPMD and OSCC. The present study indicates a role for serum IgA in OSCC while salivary IgA has potential as a prognostic indicator in OPMD and OSCC.

Financial support and sponsorship

This study was financially supported by Nitte University Research Grant.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Feller L, Lemmer J. Oral squamous cell carcinoma: Epidemiology, clinical presentation and treatment. J Cancer Ther 2012;3:263-8.
2. Massano J, Regateiro FS, Januário G, Ferreira A. Oral squamous cell carcinoma: Review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:67-76.
3. Srivastava R, Sharma R, Mishra S, Singh RB. Biochemical and molecular biological studies on oral cancer: An overview. Open Nutraceuticals J 2011;4:180-8.
4. Mathur PT, Dayal PK, Pai K. Correlation of clinical patterns of oral squamous cell carcinoma with age, site, sex and habits. J Indian Acad Oral Med Radiol 2011;23:81-5.
5. Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007;36:575-80.
6. van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol 2009;45:317-23.
7. Hosagadde S, Dabholkar J, Virmani N. A clinicopathological study of oral potentially malignant disorders. J Head Neck Physicians Surg 2016;4:29-34.
8. Mortazavi H, Baharvand M, Mehdipour M. Oral potentially malignant disorders: An overview of more than 20 entities. J Dent Res Dent Clin Dent Prospects 2014;8:6-14.
9. 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.
10. Lasisi TJ, Yusuf BO, Lasisi OA, Akang EE. Salivary and serum IgA evaluation of patients with oro-facial squamous cell carcinoma. Int J Otolaryngol Head Neck Surg 2013;2:42-5.
11. Parveen S, Taneja N, Bathi RJ, Deka AC. Evaluation of circulating immune complexes and serum immunoglobulins in oral cancer patients – A follow up study. Indian J Dent Res 2010;21:10-5.
12. Neville BW, Day TA. Oral cancer and precancerous lesions. CA Cancer J Clin 2002;52:195-215.
13. Byakodi R, Byakodi S, Hiremath S, Byakodi J, Adaki S, Marathe K, *et al.* Oral cancer in India: An epidemiologic and clinical review. J Community Health 2012;37:316-9.
14. Sridharan G. Epidemiology, control and prevention of tobacco induced oral mucosal lesions in India. Indian J Cancer 2014;51:80-5.
15. Jadhav KB, Gupta N. Clinicopathological prognostic implicators of oral squamous cell carcinoma: Need to understand and revise. N Am J Med Sci 2013;5:671-9.
16. 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.
17. Brown AM, Lally ET, Frankel A, Harwick R, Davis LW, Rominger CJ. The association of the IGA levels of serum and whole saliva with the progression of oral cancer. Cancer 1975;35:1154-62.
18. Shilpashree HS, Sarapur S. Evaluation of salivary immunoglobulin A levels in tobacco smokers and patients with recurrent aphthous ulcers. J Nat Sci Biol Med 2012;3:177-81.
19. Phillips AC, Carroll D, Drayson MT, Der G. Salivary immunoglobulin A secretion rate is negatively associated with cancer mortality: The West of Scotland Twenty-07 Study. PLoS One 2015;10:e0145083.
20. Taneja L, Bagewadi A, Keluskar V, Arya V. Estimation of major immunoglobulin levels in patients with oral submucous fibrosis. J Craniomaxillary Dis 2015;4:16-20.
21. Krasteva A, Aleksiev E, Ivanova A, Altankova I, Bocheva T, Stanimirov P, *et al*. Salivary components of treated cancer patients and patients with precancerous lesions. J IMAB 2008;14:41-4.
22. Jafarzadeh A, Sadeghi M, Karam GA, Vazirinejad R. Salivary IgA and IgE levels in healthy subjects: Relation

to age and gender. Braz Oral Res 2010;24:21-7.
23. Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, *et al.* Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clin Exp Immunol 2008;151:42-50.
24. Khan SF, Katti G, Baba I, Khan N. Age-related changes of salivary IgA among healthy subjects. J Indian Acad Oral Med Radiol 2015;27:203-6.
25. Butchibabu K, Swaminathan M, Kumar S, Koppolu P, Kiran K, Muralikrishna T. Estimation of salivary immunoglobulin A levels in gingivitis and chronic periodontitis patients before and after phase I periodontal therapy. J NTR Univ Health Sci 2014;3 Suppl S1:23-7.
26. Giuca MR, Pasini M, Tecco S, Giuca G, Marzo G. Levels of salivary immunoglobulins and periodontal evaluation in smoking patients. BMC Immunol 2014;15:5.