Salivary IgA and Total Protein in Human Subjects with Oral Lichen Planus and Squamous Cell Carcinoma

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ABSTRACT

Purpose: The purpose of this study was to compare salivary immunoglobulin A (IgA) and total protein (TP) in patients suffering from oral lichen planus (OLP) and oral squamous cell carcinoma (OSCC), against healthy control.

Methods: In a case-control study, 20 patients with OSCC, 30 patients suffering from OLP and 50 non-involved subjects were enrolled. The mean stimulated and unstimulated saliva IgA and TP levels were assayed by the Biuret method and immunoturbidometry respectively. Statistical analysis of the ANOVA was performed.

Results: The mean concentration of stimulated and unstimulated saliva IgA and TP were significantly higher in both OLP and OSCC patients compared to control. Additionally TP, but not IgA, was higher in patients with OSCC compared with OLP patients d more salivary TP than OLP patients.

Conclusion: Results suggest that salivary TP but not IgA is higher in OSCC patients than OLP patients.

Keywords: Total protein; IgA; oral lichen planus; Oral squamous cell carcinoma; saliva

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INTRODUCTION

Lichen planus is a chronic inflammatory disease which afflicts skin and mucosa. The prevalence of oral lichen planus (OLP) is relatively common worldwide (0.5-2.2%), and frequently presents in middle-aged patients, biased towards women in a ratio of 2 to 1 (1). Despite many studies, the etiology of OLP is very complex and not completely understood, but it is accepted that the immune system, especially auto-cytotoxic CD8 apoptosis, can cause this chronic inflammation (2). Oral antigen presentation could have either an endogenous or exogenous origin. The antigen trigger is accompanied by mixed inflammatory responses, constituting mainly of T-cells, mast cells, macrophages, cytotoxic molecules and associated cytokines. In recent decades it has become clear that OLP lesions have a risk of malignancy, and can develop into oral squamous cell carcinoma (OSCC), making this the major concern. Topical corticosteroids and systemic corticosteroids are the most common agents for treatment of OLP but they are not satisfactory (3, 4).

OSCC includes more than 90% of all oral cancers, and usually affects people aged between 50 and 60 years of age. It can appear in normal mucosa or develop from some precancerous disorder (3). Despite advances in therapeutic methods, mortality rate is about 50% at the present time (5).

The majority of previous studies on diagnosis of these diseases have been focused on serum, and very few on salivary parameters. Saliva is a complex fluid and several situations can modify its composition (6). Saliva analysis can be helpful in early diagnosis of several diseases and its importance in laboratory tests has been proven (7, 8). Additionally, its collection is non-invasive and simple, and does not require complex training (3).

Total Protein and Immunoglobulin A (IgA) are routine tests performed on patients suspected of any pathology, and therefore any significant changes in them can aid in early diagnosis. IgA is the principal immunoglobulin of the oral fluid, includes 85% of all salivary Ig (9), and has a critical role in mucosal immune (10). It has been shown that unstimulated salivary IgA is higher in OSCC patients (11). Total Protein (TP) is a basic factor in saliva and saliva TP is higher in OSCC patients than in healthy controls (12-14). We have found no report on salivary Total Protein and IgA differences between OSCC and OLP patients. Therefore, this study, comparing salivary levels of Total Protein and IgA among OSCC patients, OLP patients and healthy control, was conducted to help assess their role as diagnostic markers for detecting OLP and OSCC, and monitoring the progression of the diseases.

METHODS

Ethical approval

This study conformed to the regulations of the Ethics Committee of Tehran University of Medical Sciences (TUMS). Written consent was obtained from each participant in our study.

Subjects

The subjects of this case-control study consisted of 30 patients with OLP (5 men/25 women, age range 32-73 years), 20 patients with OSCC (13 men/7 women, age range 28-70 years) and 50 healthy people (15 men/35 women, age range 27-69 years) as control.

Patients with OLP were selected from the Department of Oral Medicine, Faculty of Dentistry, TUMS. Clinic-pathological inclusion criteria were based on a modified WHO definition (15). Excluded from the study were: subjects with systemic diseases, or consuming

drugs; subjects with any sign of dysplasia, licheniod reactions, or any other oral disease; or who had received treatment for OLP within one month of the start of this study.

Patients with OSCC were selected from the Cancer Department of Emam Khomeini Hospital of TUMS, and diagnosis was confirmed with histopathological discoveries. Patients with systemic diseases, any other oral disease, or who had received chemotherapy or radiotherapy within six month of the start of this study, or who had had surgery of the exact lesion, were excluded.

Fifty volunteers from staff and entourage of patients of TUMS were enrolled in this study as controls. Any potential participant with more than 3 mm periodontal pocket was also excluded.

Saliva collection

The procedure of the study was explained to the participants, and saliva samples were collected. To avoid circadian variations, all samples were collected in the morning between 10 a.m. and 12 p.m., after participants had fasted for about 2 hours. Collection For collection of unstimulated saliva, participants assumed a resting position for 10-15 minutes, were requested to collect the saliva in their mouth without any stimulation, and then to spit into dry plastic vials. Subsequently, subjects were requested to chew a similar piece of paraffin, and then to spit the stimulated saliva into another dry plastic vial. The samples were centrifuged for 10 minutes in 2000g and then frozen at -20°c for dispatch to laboratory for analysis.

Saliva analysis

Whole saliva was assessed colorimetrically by a spectrophotometer and using affiliated kits (Pars Azmoon, Karaj, Iran) for the concentrations of total protein and IgA. Total protein concentration was measured by the Biuret method, using bovine serum albumin as a standard and IgA by immunoturbidometry.

Statistical analysis

Statistical analysis was done with SPSS software. Comparison of data among groups was done using ANOVA followed by Student-Newman-Keuls Test. $P < 0.05$ was considered significant in this study. The data were explained as mean ± SEM.

RESULTS

There were no significant differences in stimulated and unstimulated saliva IgA or TP between male and female in the control, OLP and OSCC groups.

A one-way ANOVA indicated that the mean stimulated ($F_{(2,97)}=14.5$, $P=0.01$) and unstimulated ($F_{(2,97)}=5.1$, $P=0.034$) saliva IgA were significantly different among groups (Fig. 1a,b). Post-hoc analysis showed that stimulated and unstimulated IgA concentrations were significantly higher in OLP and OSCC patients compared to the control groups; but there were no significant differences in stimulated and unstimulated IgA concentrations between OSCC and OLP patients ($P > 0.05$).

The mean stimulated ($F_{(2,97)}=38.4$, $P=0.0001$) and unstimulated ($F_{(2,97)}=101.1$, $P=0.0001$) saliva total protein were significantly different among groups (Fig. 2). Post-hoc analysis showed that stimulated and unstimulated total protein concentrations were significantly higher in OLP and OSCC patients compared to the control groups and also higher in OSCC compared to OLP patients ($P < 0.05$).

Also, there was no significant difference in stimulated and unstimulated salivary IgA and TP concentrations between the erosive and reticular form of OLP ($p>0.05$) (Table.1).

DISCUSSION

OLP is a mucocutaneous disease. Oral lesions in OLP are more resistant and complicated compared to extra oral lesions, and may transform to malignancy. Importantly, the pathogenesis of OLP is unknown, therefore preventive planning cannot be efficient. Due to the importance of OLP as a relatively common pre-neoplastic inflammatory lesion, evaluation of salivary factors may help to reveal the mechanism of transformation of OLP to OSCC, and the probable correlation between them.

The major concern of patients with OLP is transformation to malignancy; therefore efforts have been focused on discovering the modality that can predict the lesions prone to transformation. In our previous studies we measured the levels of salivary p53 (16), 8-OHdG, malondialdehyde (MDA) as the end product of the oxidation of polyunsaturated fatty acids, and total antioxidant capacity (TAC) in OSCC as compared to OLP and controls (2).

Specific antibody systems, including salivary IgA may have an important role as a first line in protection against pathogens in oral diseases that colonize and invade mucosal surfaces (8). In fact the importance and role of salivary immunoglobulin in clinical course and immunopathogenesis of chronic inflammatory diseases has not been reviewed yet (8). Our results showed that there was no significant difference in IgA level between OLP and OSCC whether in stimulated or unstimulated saliva. However, stimulated and unstimulated salivary IgA levels in OLP and OSCC were significantly higher compared to controls. The elevated level of salivary IgA in OLP and OSCC may be the protective effects of IgA in these patients.

Some studies have been done on the role of salivary immunoglobulin in OLP, but they are insufficient for understanding the importance of salivary immunoglobulin in the pathogenesis

of mucosal diseases. Elevation of IgA concentration in cancers reported in our and other studies may indicate a local antibody against tumor (11).

In our study, IgA concentration in OSCC and OLP (both reticular and erosive forms), whether in stimulated or unsimulated saliva, increased equally. From these results, it seems that the reaction of this immunoglobulin to the cancers and inflammatory diseases is similar.

Sistig et al evaluated unstimulated salivary IgA level in OLP (17). The result of unstimulated salivary IgA in OLP was significantly higher than controls, conforming to our study. They declared that this elevation can show the importance of salivary immunoglobulin in OLP pathogenesis. However, this is not supported by Gandara et al, who report that the difference in salivary IgA levels is not significant between lichen planus patients and controls (18).

Brown et al evaluated salivary IgA in OSCC and found that unstimulted salivary IgA in OSCC was significantly higher than controls (11), consistent with our study. The inference of the researcher was that the increase of IgA may be a local defense against tumor (11). In another study by de Souza et al, unstimulated salivary IgA in OSCC was significantly lower than controls, contrary to our and other studies. They pointed that their result could be due to the method of saliva collection, tobacco consumption or stress in these patients (19).

In our result, stimulated and unstimulated salivary TP levels in OSCC were significantly higher than in OLP, which in turn was higher than in controls. As wound and cellular shedding usually exists in OSCC, and also biomarkers such as tumor suppressors, oncogenes and protooncogenes increase in OSCC, they may explain the elevated levels of TP in OSCC compared to OLP.

Sanjay et al showed that unstimulated salivary TP in OSCC was significantly higher than controls. The researcher declared that the role of TP in cancers is unknown and more research

with bigger samples is needed (20). In another study on OSCC, unstimulated salivary TP was significantly lower compared to controls. Researcher opinion was that this lower level of TP in OSCC is due to the reduction of protein consumption in those patients (21). Shipitzer et al indicated that unstimulated salivary TP was significantly higher but IgA was lower in OSCC than controls (22). In this study, in addition to unstimulated salivary TP and IgA, stimulated salivary TP and IgA between OLP and controls and OSCC and controls were evaluated. We also compared these two factors in stimulated and unstimulated saliva between two type of OLP (erosive and reticular), and we showed that there were no significant differences in TP and IgA levels whether in stimulated or unstimulated saliva between reticular and erosive forms of OLP.

Since OLP is a chronic inflammatory autoimmune disease, a look at studies into some other inflammatory diseases may be helpful. In research done on patients with Sjögren's syndrome, salivary IgA and TP levels were significantly higher compared to controls (23). Research on patients with rheumatoid arthritis showed their salivary IgA level was significantly higher than controls, too (24). And in research on patients with diabetes mellitus, their salivary IgA and TP were significantly higher than controls (25).

CONCLUSION

OSCC Patients have more salivary TP than OLP patients.

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REFERENCES

1. Agha-Hosseini F, Moslemi E, Mirzaii-Dizgah I. Comparative evaluation of low-level laser and CO2 laser in treatment of patients with oral lichen planus. Int J Oral Maxillofac Surg 2012; 41(10):1265-9
2. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. J Oral Pathol Med 2012; 41(10):736-40.
3. Taghavi Zenouz A, Mehdipour M, Attaran R, Bahramian A, Emamverdi Zadeh P. Squamous cell carcinoma arising from an oral lichenoid lesion: a case report. J Dent Res Dent Clin Dent Prospects 2012; 6(1):29-32.
4. Georgakopoulou EA, Achtari MD, Achtaris M, Foukas PG, Kotsinas A. Oral lichen planus as a preneoplastic inflammatory model. J Biomed Biotechnol 2012:759626. doi: 10.1155/2012/759626.
5. Braam PM, Roesink JM, Raaijmakers CP, Busschers WB, Terhaard CH. Quality of life and salivary output in patients with head-and-neck cancer five years after radiotherapy. Radiat Oncol 2007; 2:3.
6. Aps JK, Martens LC. Review: The physiology of saliva and transfer of drugs into saliva. Forensic Sci Int 2012; 150(2-3):119-31.
7. Agha-Hosseini F, Mirzaii-Dizgah I, Ghavamzadeh L, Ghavamzadeh A, Tohidast-Acrad Z. Effect of pilocarpine hydrochloride on unstimulated whole saliva flow rate and composition in patients with chronic graft-versus-host disease (cGVHD). Bone Marrow Transplant 2007; 39(7):431-4.

8. Branco-de-Almeida LS, Alves CM, Lopes FF, Pereira Ade F, Guerra RN, Pereira AL. Salivary IgA and periodontal treatment needs in diabetic patients. Braz Oral Res 2011; 25(6):550-5.

9. van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Res 2004; 38(3):247-53.

10. Otsuki T, Shimizu K, Iemitsu M, Kono I. Salivary secretory immunoglobulin A secretion increases after 4-weeks ingestion of chlorella-derived multicomponent supplement in humans: a randomized cross over study. Nutr J 2011; 10:91. doi: 10.1186/1475-2891-10-91.

11. 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(4):1154-62.

12. Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci 2010; 52(3):359-68.

13. Franzmann EJ, Reategui EP, Pereira LH, Pedroso F, Joseph D, Allen GO, et al. Salivary protein and solCD44 levels as a potential screening tool for early detection of head and neck squamous cell carcinoma. Head Neck. 2012 May;34(5):687-95. doi: 10.1002/hed.21810.

14. Dhakar N, Astekar M, Jain M, Saawarn S, Saawarn N. Total sialic acid, total protein and total sugar levels in serum and saliva of oral squamous cell carcinoma patients: A case control study. Dent Res J 2013; 10(3): 343–47.

15. van der Meij EH, van der Waal I. Lack of clinicopathologic correlation in the diagnosis of oral lichen planus based on the presently available diagnostic criteria and suggestions for modifications. J Oral Pathol Med 2003; 32: 507-512. doi:10.1034/j.1600-0714.2003.00125.x

16. Thomas L. Clinical Laboratory Diagnostics, 1st edn. TH-Books verlagsgesellschaft: Frankfurt. 1998.

17. Sistig S, Vucićević-Boras V, Lukac J, Kusić Z. Salivary IgA and IgG subclasses in oral mucosal diseases. Oral Dis 2002; 8(6):282-6.

18. Gandara BK, Izutsu KT, Truelove EL, Mandel ID, Sommers EE, Ensign WY. Sialochemistry of whole, parotid, and labial minor gland saliva in patients with oral lichen planus. J Dent Res. 1987 Nov; 66 (11):1619-22.

19. de Souza RM, Lehn CN, Denardin OV. Serum and salivary immunoglobulin A levels in patients with cancer of the mouth and oropharynx. Rev Assoc Med Bras 2003; 49(1):40-4.

20. Sanjay PR, Hallikeri K, Shivashankara AR. Evaluation of salivary sialic acid, total protein, and total sugar in oral cancer: a preliminary report. Indian J Dent Res 2008; 19(4):288-91.

21. Fuchs PN, Rogić D, Vidović-Juras D, Susić M, Milenović A, Brailo V, et al. Salivary analytes in patients with oral squamous cell carcinoma. Coll Antropol 2011; 35(2):359-62.

22. Shpitzer T, Bahar G, Feinmesser R, Nagler RM. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol 2007; 133(9):613-7.

23. Hazi-Mihailović M, Janković L, Cakić S. Circulating immune complexes, immunoglobulin G, salivary proteins and salivary immunoglobulin A in patients with Sjögren's syndrome. Srp Arh Celok Lek 2009; 137(3-4):134-9.

24. Ben-Aryeh H, Nahir M, Scharf Y, Gutman D, Laufer D, Szargel R. Sialochemistry of patients with rheumatoid arthritis. Electrolytes, protein, and salivary IgA. Oral Surg Oral Med Oral Pathol 1978; 45(1):63-70.

25. Yavuzyilmaz E, Yumak O, Akdoğanli T, Yamalik N, Ozer N, Ersoy F, et al. The alterations of whole saliva constituents in patients with diabetes mellitus. Aust Dent J 1996; 41(3):193-7.

Table 1: Stimulated and unstimulated saliva IgA and total protein (TP) concentrations in patients with oral lichen planus (OLP) and controls

	controls	OLP- Reticular (n=21)	OLP-Erosive (n=9)
Stimulated saliva IgA (mg/dl)	26.15±1.40	30.88 ± 1.95*	32.22 ± 2.32 *
Unstimulated saliva IgA (mg/dl)	22.48 ± 1.23	34.04 ± 2.23*	31.44 ± 4.11*
Stimulated saliva TP (mg/dl)	183 ± 19	549 ± 66 *	632 ± 84 *
Unstimulated saliva TP (mg/dl)	279 ± 27	638 ± 38 *	580 ± 40 *

Data are expressed as mean ± SEM. * Different from controls, $P<0.05$

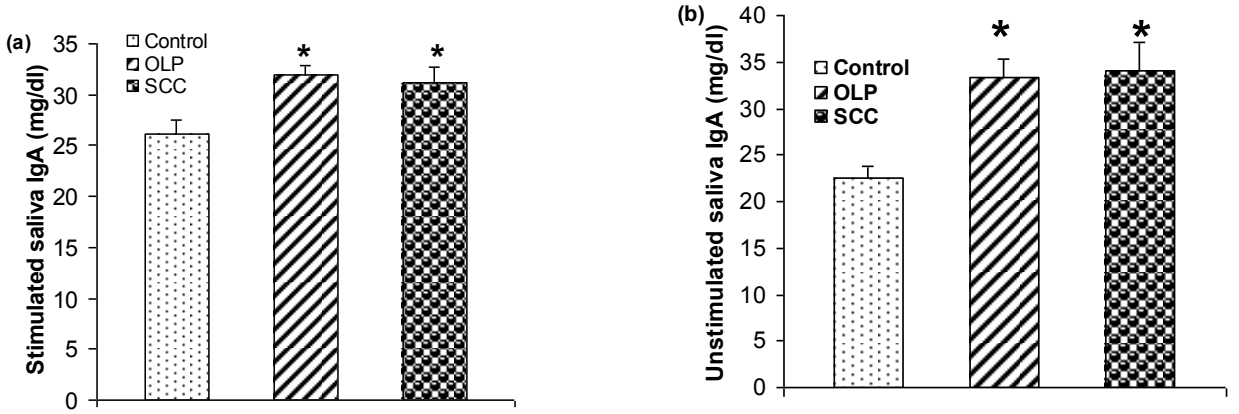

Fig. 1: stimulated (a) and unstimulated (b) saliva concentration of IgA in patients with squamous cell carcinoma (SCC), oral lichen planus (OLP) and controls. Data are expressed as mean±SEM.*Different from controls, P<0.05

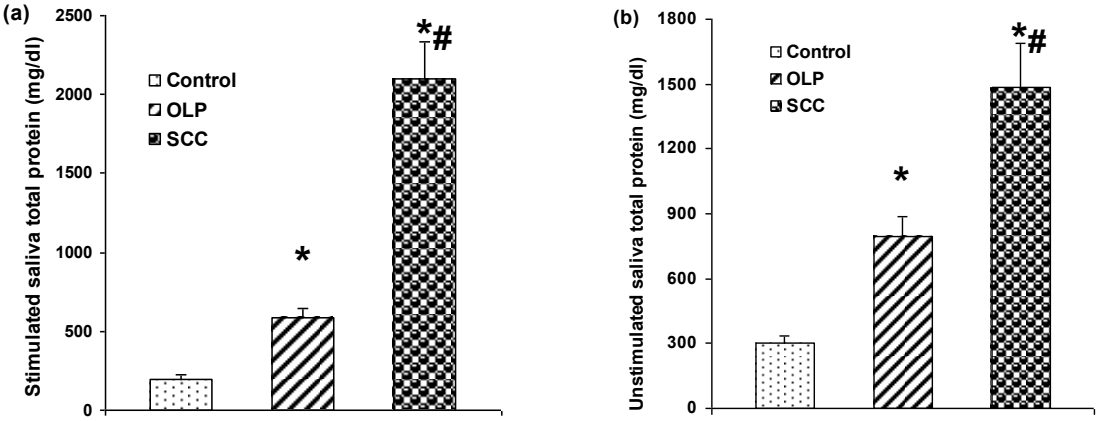

Fig. 2: stimulated (a) and unstimulated (b) saliva concentration of total protein in patients with squamous cell carcinoma (SCC), oral lichen planus (OLP) and controls. Data are expressed as mean ± SEM.* Different from controls; #different from OLP patients, P<0.05.