Original Article

Expression of salivary levels of S100A7 in oral submucous fibrosis and oral leukoplakia

Razwia Fathima[1], Raghini Ramamoorthi[1], Sivakumar Gopalakrishnan[2], Vijayashree P. Jayaseelan[3], Sivakumar Muniapillai[1]

[1]Department of Oral Pathology and Microbiology, Madha Dental College and Hospital, Kundrathur, [2]Department of Oral Pathology and Microbiology, Madha Dental College and Hospital, Kundrathur, and Research Scholar, Clinical Genetics Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, [3]Associate Professor, Clinical Genetics Lab, Centre for Cellular and Molecular Research, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India

Abstract

Aim: The aim of the study is to evaluate the expression of S100A7 levels in saliva of oral sub-mucous fibrosis, oral leukoplakia patients, and healthy control.

Materials and Methods: The study comprised of saliva samples from 15 patients each with clinically diagnosed oral sub-mucous fibrosis, oral leukoplakia, and healthy control. Salivary S100A7 levels were estimated using Enzyme-Linked Immunosorbent Assay. Statistical analysis was performed using SPSS. The significance level is fixed at 5% ($\alpha = 0.05$). To compare the mean values of concentration between the disease group oral leukoplakia (OL) and oral submucous fibrosis (OSMF) and control, one-way analysis of variance was used followed by a *post hoc* test for multiple pairwise comparisons.

Results: The results of the study indicated a statistically significant increase in the salivary S100A7 level among the OSMF and OL when compared with the control group. When a pairwise comparison was done between OSMF with a control group and leukoplakia with a control group, a statistically significant difference was observed, subsequently while comparing OSMF with leukoplakia, and no statistically significant difference was observed.

Conclusion: Results from this study demonstrated increased S100A7 levels in OSMF and OL when compared with control group. This indicated that salivary S100A7 can be used as an adjunctive marker to identify patients at risk of progression into oral squamous cell carcinoma (OSCC).

Keywords: ELISA, oral leukoplakia, oral sub mucous fibrosis, oral squamous cell carcinoma, S100A7

INTRODUCTION

Oral potentially malignant disorders (OPMDs) are a set of oral mucosal lesions associated with a higher likelihood of cancer. These disorders comprise diseases of varying risk factors, clinical features, and histological types.[1] Potentially malignant disorders are considered precursors for the development of oral squamous cell carcinoma. Precise

Access this article online

Quick Response Code:

Website:
https://journals.lww.com/JPAT/

DOI:
10.4103/jomfp.jomfp_113_23

How to cite this article: Fathima R, Ramamoorthi R, Gopalakrishnan S, Jayaseelan VP, Muniapillai S. Expression of salivary levels of S100A7 in oral submucous fibrosis and oral leukoplakia. J Oral Maxillofac Pathol 2024;28:84-9.

diagnosis and prompt treatment of OPMDs are essential for preventing the malignant development of oral lesions.[2]

The range of OPMDs comprises oral leukoplakia, erythroleukoplakia, erythroplakia, oral sub-mucous fibrosis, oral lichen planus, lichenoid reactions, palatal lesions in reverse smokers, oral lupus erythematosus, graft vs. host disease, epidermolysis bullosa, and dyskeratosis congenita.[3]

Leukoplakia now affects 4.11% of the world's population, with a surge in Asian populations.[4] It occurs above the age range of 30-40 years. The estimated annual risk of cancer transformation varies within the range of 2%-3% and even higher.[5] Prevalence increases with an increase in age and shows male predominance in different parts of India.[6] [Figure 1].

OSMF is commonly observed in Indian young adults of the 20-40 age range with a male to female ratio of 32.1:1. OSF patients are 19 times more at risk of developing OSCC than healthy people. OSF has a malignant transformation of 2%-8%[7] [Figure 2].

Early detection of OPMDs is essential for reducing the mortality and morbidity of OSCC. Identification of OPMDs developing into malignancy remains a challenge, as the malignant transformation of OPMD is not consistent. Histopathological diagnosis has limited prognostic value in assessing the progression of OPMDs into invasive carcinoma. The development of an alternative method is important in predicting the fatality of suspicious lesions. Hence, the necessity of biomarkers has been indicated for screening and diagnosis of OPMD and OSCC.[8,9]

Saliva, a biological fluid can be used to monitor the disease as a diagnostic tool. It was suggested as a suitable biological sample due to its noninvasive collection. It is also effective in detecting the biomarkers of recent technologies.[10]

S100A7 (Psoriasin), a member of the S100 gene family, was first detected as an 11.4 kDa protein in squamous cells of the epidermis isolated from psoriasis-affected skin. It shares chromosomal proximity and homology with other S100 gene family members.[11]

S100A7 has a significant role in the regulation of cell cycle, inflammation, transcription, proliferation, and survival in numerous malignancies of epithelium such as head and neck, breast, bladder, lung, skin, esophageal, colorectal, and gastric cancers. Overexpression of S100A7 serves as a biomarker in assessing cancer progression risk from oral dysplastic lesions.[12]

MATERIALS AND METHOD

Study design
A case-control study was carried out among patients reporting to the Department of Oral Pathology and Microbiology after approval by the institutional ethical committee for the year 2020-2021. Saliva samples were obtained after explaining the entire procedure to the study participants.

Study participants
The study includes 15 patients clinically diagnosed with oral sub-mucous fibrosis and 15 patients clinically diagnosed with oral leukoplakia with the habit of areca nut and smoking. To compare, 15 clinically healthy participants were included.

Saliva sample collection
After clinical examination, unstimulated whole saliva (2 mL) was collected by the passive drool technique. Patients are seated in comfort with heads inclined forward. Patients are asked to pool saliva within the mouth without any muscular movement stimulation. Patients are then instructed to spit the saliva into a graduated sterile container. Samples are transported together with an ice pack in a ventilated
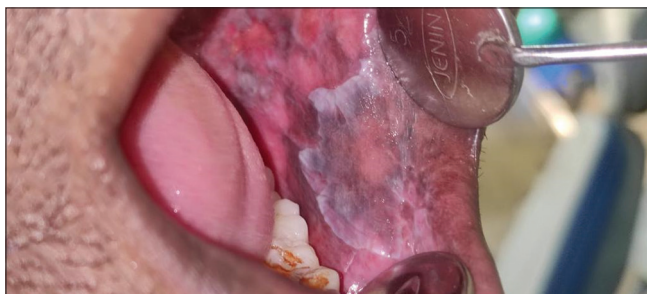

Figure 1: Leukoplakia in left buccal mucosa

Figure 2: OSMF in right buccal mucosa

polystyrene box at a temperature of -4°C. Samples are then stored in a deep freezer at -80°C for further analysis by enzyme linked immunosorbent assay (ELISA).

Assay procedure

The Human S100A7 Elisa kit quantitates S100A7 in samples by employing a two-site sandwich ELISA. All the reagents must be prepared before starting assay procedure [Figure 3]. A required number of wells were inserted in the frames. Standard wells and testing sample wells were assigned. Diluted standard 50 µL is added to the standard well. Sample diluent 40 µL was added first to the testing sample well. Then 10 µL of the sample was added to the testing sample well. Wells were incubated after covering with a plate cover for 45 minutes at 370°C. Then each well was aspirated and washed for 1-3 minutes, for a total of four times. Each well was washed by using a manifold dispenser with wash buffer (250 µL). Any leftover buffer was aspirated at the final wash. Horseradish peroxidase (HRP)-conjugated detection antibody 50 µL was added to each well. Wells were incubated after covering with plate cover for 30 minutes at 370°C. Aspiration process was repeated five times as done earlier. Then 50 µL of chromogen solution A and 50 µL of chromogen solution B were added to each well. Gently mixed and incubated for 15 minutes at 370°C [Figure 4]. Stop solution 50 µL was added to each well. The colour of the wells changed from blue to yellow [Figure 5]. Optical density (OD) was read at 450 nm within 15 minutes using a microtiter plate reader [Figure 6]. The concentration of S100A7 was calculated.

Statistical analysis

To analyse the data, SPSS (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019) is used. The significance level is set at 5% ($\alpha = 0.05$). The normality tests such as Kolmogorov-Smirnov and Shapiro-Wilks tests results reveal the study followed a normal distribution. Therefore, to analyze the data, a parametric test was applied. Descriptive statistics were done to assess the mean among the study variables and Chi-square test was done to assess the mean difference among the age. To compare the mean values of concentration between the disease group (OL and OSMF) and control, one-way analysis of variance was used followed by multiple pairwise comparisons by *post hoc* test.

RESULTS

The mean (± standard deviation) age of oral leukoplakia, oral sub-mucous fibrosis, and control were 54.80 ± 6.81, 45.26 ± 9.13, and 25.40 ± 2.79, respectively. On comparing the mean age by Chi-square test between the disease groups and control, *P* value is. 012 (statistically significant difference observed) [Table 1 and Figure 1].

Table 1: Age distribution among the study groups

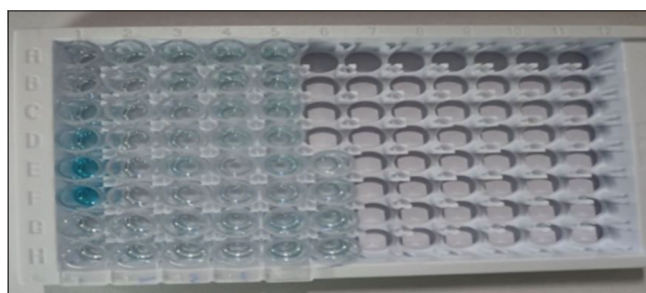| Study groups	Mean age (years)	Standard deviation	*P*
Control	25.40	2.79	0.012*
Leukoplakia	54.80	6.81	
OSMF	45.26	9.13	

Figure 3: Contents of S100A7 ELISA kit

Figure 4: Blue color change in ELISA wells after adding chromogen solution
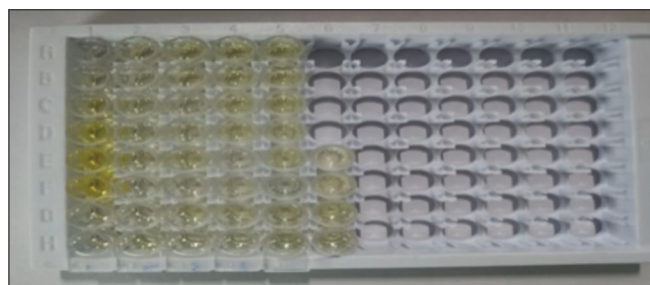

Figure 5: Yellow color change after adding stop solution

Figure 6: Microtitre plate reader

Salivary S100A7 concentration in the control group ranges from 12.12-15.22 in nine patients, 22.62-24.43 in two patients, and 40.62-54.19 in four patients. Salivary S100A7 concentration in leukoplakia ranges from 20.08-24.13 in two patients, 26.88-37.95 in six patients, and 41.14-55.22 in seven patients. Salivary S100A7 in OSMF ranges from 32.04-39.31 in three patients and 40.36-72.23 in 12 patients [Table 2].

S100A7 levels in saliva of oral leukoplakia, oral sub-mucous fibrosis, and controls were measured using ELISA test. The mean value of salivary S100A7 for OL is 38.2065 ng/L ± 10.55721, OSMF is 45.8105 ng/L ± 9.39788, and control is 24.1945 ng/L ± 6.08004.

The one-way analysis of variance test showed mean difference was statistically highly significant with *P* value of <0.01 [Table 3 and Figure 2].

A multiple pairwise comparison by *post hoc* test was made between the leukoplakia, OSMF, and control groups. On comparing leukoplakia and OSMF with the control group, a statistically significant difference with the *P* value of <0.01 was observed. Subsequently, no statistically significant difference was observed when a pairwise comparison was made between leukoplakia and OSMF [Table 4].

DISCUSSION

Current molecular pathogenesis postulates that the cumulation of genetic and epigenetic changes leads to the development of cancer in a clonal cell population. These genotypic changes lead to phenotypic alterations in crucial cellular functions, like resistance to apoptosis, neo-angiogenesis, increased proliferation, and distant metastasis. Mechanism of genetic and epigenetic alteration include genomic instability, deletion, amplification, mutation, and methylation. Cancer development has been directly linked to these genetic aberrations.[3]

In this study, the mean age of oral leukoplakia is 55 years. This finding was consistent with the study performed by Mathew AL *et al.* (2008) who explained that the mean age of leukoplakia was in the 41-60 years age group. In the case of OSMF, mean age is found to be 45 years which was also similar to the findings of Mathew AL *et al.* (2008) who explained that the high prevalence of OSMF is in the age group of 41-60 years.[13]

In this study, mean salivary S100A7 levels among 15 oral leukoplakia patients were estimated as 38.2065 ng/L. These results have shown statistically significant elevated levels of S10 0A7 when compared with the control group whose S100A7 level was estimated as 24.1945 ng/L. This study was in accordance with the finding done by Sivadasan *et al.* (2019) in which he analyzed increased salivary S100A7 levels in dysplastic leukoplakia when compared with healthy control.[14]

S100 proteins exhibit a wide variety of functions such as regulation of transcriptional factors, cell proliferation,

Table 2: Salivary s100a7 concentration among study groups

Study group	Concentration range (ng/L)	No. of patients	Total no. of patients
Control	12.12-15.22	9	15
	22.62-24.43	2	
	40.62-54.19	4	
Leukoplakia	20.08-24.13	2	15
	26.88-37.95	6	
	41.14-55.22	7	
OSMF	32.04-39.31	3	15
	40.36-72.23	12	

Table 3: One-way ANOVA to compare mean salivary s100a7 levels among the study groups

Study groups	Mean	Std. error of mean	Std. deviation	Variance	Range	*F*	*P*
Control Group	24.1945	1.56986	6.08004	36.967	19.26		
Leukoplakia Group	38.2065	2.72586	10.55721	111.455	35.14	22.854	<0.01*
OSMF Group	45.8105	2.42652	9.39788	88.320	40.18		

Table 4: Pairwise comparison of concentration among the study groups

Study groups	Mean difference	Std. error	*P*	95% confidence interval	
				Lower bound	Upper bound
Control					
Leukoplakia	14.01200*	3.24374	<0.01*	-21.8926	-6.1314
OSMF	21.61593*	3.24374	<0.01*	-29.4966	-13.7353
Leukoplakia					
Control	14.01200*	3.24374	<0.01*	6.1314	21.8926
OSMF	7.60393	3.24374	0.061	-15.4846	0.2767
OSMF					
Control	21.61593*	3.24374	<0.01*	13.7353	29.4966
Leukoplakia	7.60393	3.24374	0.061	-0.2767	15.4846

apoptosis, calcium homeostasis, cell invasion and motility, cytoskeleton interactions, autoimmunity, and inflammation. S100A7 protein is encoded by 1q21.3 loci. It is the main locus in oral cancer that encodes for the utmost number of differentially expressed proteins. These features represent that S100A7 could be a potential tumor biomarker.[14]

In the present study, mean salivary S100A7 levels among 15 OSMF patients were estimated as 45.8105 ng/L. These results have revealed statistically significant elevated levels of S100A7 when compared with the control group whose S100A7 levels were estimated as 24.1945 ng/L. This study agrees with the finding done by Raffat MA *et al*. (2018) in which he determined level of S100A7 in saliva may be one of the diagnostic biomarkers for assessing inflammatory changes in OSMF.[15]

Recent research has shown that S100A7 can bind with receptor for advanced glycation end products (RAGEs) which are mainly participated in inflammatory reactions. RAGE consecutively activates NF-kB protein complex which mediates the production of proinflammatory cytokine. Besides, S100A7 increases the ROS level intracellularly in keratinocytes, implying a feedback loop in which S100A7 is stimulated by ROS and increases hypoxia.[15] It could be the possible pathogenesis for increased S100A7 levels in OSMF. Thus, the detection of S100A7 levels in saliva may provide significant information about oral premalignant conditions including leukoplakia and OSMF.

Furthermore, pairwise comparison between OL, OSMF, and control group has shown statistically significant differences in OL–control group and OSMF–control group. Elevated S100A7 levels were observed in OSMF when compared to leukoplakia but statistically, a significant difference was not obtained. Elevated levels of S100A7 in OSMF indicate that the malignant potential of OSMF is higher than leukoplakia.

S100A7 have been linked to tumor progression, neo-angiogenesis, and distant metastasis. It contributes to the interference between the tumor and the stromal cells, thus enhancing the aggregation of inflammatory, angiogenic, invasive, prometastatic growth factors, and cytokines. Thus, it provides a potent stimulus for tumor growth and dissemination. S100A7 is seen extracellularly under pathological conditions and can cause migration of tumor and stromal cells, and endothelial cell proliferation through communication with RAGE receptor. S100A7 expression can be regulated by microenvironmental conditions in tumor cells, such as hypoxia and high cellular density among others. In addition, the intriguing function that the S100A7 protein plays in tumor formation has led to its recognition as a novel cancer treatment target.[16] These molecular mechanisms represent that the level of S100A7 increases when there is malignant transformation of a premalignant condition.

In another study done by Raffat MA *et al*. (2019) in stage I OSMF, also showed statistically significant increased S100A7 levels in saliva when compared with the control group.[17]

Several other studies (Jou *et al*., 2014; Dey *et al*., 2015) have investigated the role of S100A7 in the saliva of OSCC patients and they suggested that S100A7 was a potent salivary biomarker for diagnosis of OSCC. S100A7 levels in OSCC were elevated in early malignancy stage and falls dramatically in the later stages of malignancy.[18,19]

A recent IHC study done by Sood *et al*. (2022) in tissue samples of OSCC and OPMD revealed a statistically significant cytoplasmic and nuclear staining pattern of S100A7 in OSCC and OPMD. However, OSCC tissues showed increased intensity of S100A7 antibody than OPMD. Thus, the role of S100A7 as a diagnostic biomarker for carcinomatous transformation is underscored.[20]

In this study, a significant difference of S100A7 was observed in OL, OSMF, and healthy control. This denotes that these patients are an increased potential for developing cancer. Findings from the present study suggest that S100A7 could be a promising diagnostic salivary biomarker in detecting a malignant change at an early stage in oral leukoplakia and OSMF patients.

CONCLUSION

Saliva samples used in this study is an easily accessible biofluid and it is a noninvasive procedure. The result of ELISA analysis revealed that salivary S100A7 is highly expressed in OMPD such as OSMF and oral leukoplakia when compared with normal control group. This indicates that salivary S100A7 could be useful as a potential diagnostic marker to identify patients at risk of progression into OSCC. Furthermore, a large-scale longitudinal study is required to validate its potential as a determinant of OSCC progression.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Lorini L, Bescós Atín C, Thavaraj S, Müller-Richter U, Alberola Ferranti M, Pamias Romero J, *et al.* Overview of oral potentially malignant disorders: From risk factors to specific therapies. Cancers (Basel) 2021;13:3696.
2. 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.
3. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:582-90.
4. Kumari P, Debta P, Dixit A. Oral potentially malignant disorders: Etiology, pathogenesis, and transformation into oral cancer. Front Pharmacol 2022;13:825266.
5. van der Waal I. Oral leukoplakia: Present views on diagnosis, management, communication with patients, and research. Current Oral Health Reports 2019;6:9-13.
6. Parlatescu I, Gheorghe C, Coculescu E, Tovaru S. Oral leukoplakia - an update. Maedica (Bucur) 2014;9:88-93.
7. Ganesh D, Sreenivasan P, Öhman J, Wallström M, Braz-Silva PH, Giglio D, *et al.* Potentially malignant oral disorders and cancer transformation. Anticancer Res 2018;38:3223-9.
8. Piyarathne NS, Rasnayake RMSGK, Angammana R, Chandrasekera P, Ramachandra S, Weerasekera M, *et al.* Diagnostic salivary biomarkers in oral cancer and oral potentially malignant disorders and their relationships to risk factors - A systematic review. Expert Rev Mol Diagn 2021;21:789-807.
9. Dikova VR, Principe S, Bagan JV. Salivary inflammatory proteins in patients with oral potentially malignant disorders. J Clin Exp Dent 2019;11:e659-64.
10. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DT. Saliva diagnostics - Current views and directions. Exp Biol Med (Maywood) 2017;242:459-72.
11. Watson PH, Leygue ER, Murphy LC. Psoriasin (S100A7). Int J Biochem Cell Biol 1998;30:567-71.
12. Kaur J, Matta A, Kak I, Srivastava G, Assi J, Leong I, *et al.* S100A7 overexpression is a predictive marker for high risk of malignant transformation in oral dysplasia. Int J Cancer 2014;134:1379-88.
13. Mathew AL, Pai KM, Sholapurkar AA, Vengal M. The prevalence of oral mucosal lesions in patients visiting a dental school in Southern India. Indian J Dent Res 2008;19:99-103.
14. Sivadasan P, Gupta MK, Sathe G, Sudheendra HV, Sunny SP, Renu D, *et al.* Salivary proteins from dysplastic leukoplakia and oral squamous cell carcinoma and their potential for early detection. J Proteomics 2020;212:103574.
15. Raffat MA, Hadi NI, Hosein M, Zubairi AM, Ikram S, Akram Z. Differential expression of salivary S100A7 in oral submucous fibrosis. Saudi Dent J 2019;31:39-44.
16. Padilla L, Dakhel S, Adan J, Masa M, Martinez JM, Roque L, *et al.* S100A7: From mechanism to cancer therapy. Oncogene 2017;36:6749-61.
17. Raffat MA, Hadi NI, Alghamdi O, Al-Aali KA, Al Deeb M, Abduljabbar T, *et al.* Expression of salivary s100a7 levels in stage i oral submucous fibrosis: A clinical and laboratory study. Asian Pac J Cancer Prev 2020;21:1115-9.
18. Jou YJ, Hua CH, Lin CD, Lai CH, Huang SH, Tsai MH, *et al.* S100A8 as potential salivary biomarker of oral squamous cell carcinoma using nanoLC-MS/MS. Clin Chim Acta 2014;436:121-9.
19. Dey KK, Sarkar S, Pal I, Das S, Dey G, Bharti R, *et al.* Erratum to: Mechanistic attributes of S100A7 (psoriasin) in resistance of anoikis resulting tumor progression in squamous cell carcinoma of the oral cavity. Cancer Cell Int 2015;15:94.
20. Sood A, Mishra D, Kharbanda OP, Chauhan SS, Gupta SD, Deo SSV, *et al.* Role of S100 A7 as a diagnostic biomarker in oral potentially malignant disorders and oral cancer. J Oral Maxillofac Pathol 2022;26:166-72.