Original Article

Evaluation of salivary alkaline phosphatase levels in tobacco users to determine its role as a biomarker in oral potentially malignant disorders

T R Menaka, G Vasupradha, S Shamala Ravikumar, K Dhivya, J Dinakaran, V Saranya

Department of Oral and Maxillofacial Pathology, Adhiparasakthi Dental College and Hospital, Melmaruvathur, Tamil Nadu, India

Abstract

Background: Elevated salivary alkaline phosphatase (S-ALP) levels have been observed in oral squamous cell carcinoma, but its status in tobacco users and in individuals with oral potentially malignant disorders (OPMDs) is less explored.

Aims and Objectives: The aims and objectives were to estimate and compare the levels of S-ALP among tobacco users, nonusers and in individuals with OPMD.

Materials and Methods: The study population comprised 42 individuals, categorized into four groups with/without tobacco usage habit and with/without lesion. 5 ml of unstimulated saliva sample was collected, centrifuged at 3000 rpm for 15 min and supernatant separated. S-ALP was estimated in the supernatant by using kinetic photometric method in an automatic analyzer.

Results: Data obtained were subjected to statistical analysis. The mean S-ALP was 18.00 IU/L for normal individuals without tobacco usage, 4.60 IU/L for smokers without lesion, 7.50 IU/L for tobacco chewers without any lesion and 64.90 IU/L for individuals with OPMD. The mean difference between the groups was statistically significant ($P < 0.001$) using Kruskal–Wallis' ANOVA. No statistically significant difference ($P > 0.05$) was obtained in the S-ALP levels between tobacco users and nonusers and between smokers and tobacco chewers, using Mann–Whitney U-test. S-ALP levels in individuals with OPMD were statistically significantly higher ($P < 0.001$) than those without lesions, with or without tobacco usage habit, using Mann–Whitney U-test.

Conclusion: We conclude that S-ALP could be used as a reliable noninvasive biomarker in monitoring OPMD.

Keywords: Biomarkers, saliva, salivary alkaline phosphatase, smokeless tobacco chewers, smokers, tobacco

Address for correspondence: Dr. S Shamala Ravikumar, Department of Oral Pathology, Adhiparasakthi Dental College and Hospital, Melmaruvathur, Tamil Nadu, India.
E-mail: shamaravi4@gmail.com
Received: 27.12.2018, **Accepted:** 27.06.2019

INTRODUCTION

Oral potentially malignant disorders (OPMDs), a terminology suggested by the World Health Organization in 2007[1] for premalignant lesions and conditions, has been reported with a high-risk percentage of malignant transformation to oral squamous cell carcinoma (OSCC). OSCC accounts for over 30% of all malignancies in the Indian population.[2] Although many etiologic factors

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_317_18

How to cite this article: Menaka TR, Vasupradha G, Ravikumar SS, Dhivya K, Dinakaran J, Saranya V. Evaluation of salivary alkaline phosphatase levels in tobacco users to determine its role as a biomarker in oral potentially malignant disorders. J Oral Maxillofac Pathol 2019;23:344-8.

have been proposed, tobacco product is a well-established etiology for the development of OPMD and OSCC.

Tissue biopsy and routine histopathology are gold standard procedures in diagnosing lesions, yet biopsy is an invasive procedure and the lesion is usually advanced at the time of diagnosis. Hence, a reliable noninvasive alternative for early detection and monitoring of OPMD in individuals at risk will greatly reduce the morbidity associated with OSCC.

In recent years, saliva which is an easily accessible oral fluid has gained acceptance as a diagnostic medium in many health conditions.[3] As it is in close contact with the lesion, it stays to be potential to detect early mucosal changes in tobacco users and in individuals with OPMD. Although few studies have been reported on using alkaline phosphatase (ALP) enzyme levels as a biomarker in serum[4] and saliva of OSCC patients, sufficient studies need to be explored in OPMD.

Hence, we undertook the present study to assess the salivary ALP (S-ALP) levels in tobacco users and in individuals with OPMD to analyze its potential as a biomarker.

Aims and objectives

The present study aimed to evaluate the levels of S-ALP among tobacco users, nonusers and in individuals with OPMDs.

The main objectives of this study were:

- To assess the levels of S-ALP in tobacco users, nonusers and in individuals with OPMD
- To compare the levels of S-ALP among tobacco users, nonusers and in individuals with OPMD.

MATERIALS AND METHODS

The study population comprised 42 individuals aged between 18 and 75 years who were categorized into four groups as follows:

- Group I – Individuals without the habit of smoking or chewing tobacco and without any lesion on intraoral examination (n = 12)
- Group II – Individuals with the habit of chewing tobacco and without any lesion on intraoral examination (n = 10)
- Group III – Individuals with the habit of smoking and without any lesion on intraoral examination (n = 10)
- Group IV – Individuals with lesion on intraoral examination with the habit of smoking/chewing tobacco (n = 10).

Inclusion criteria

For Group II, Group III and Group IV, individuals with the habit of smoking/tobacco chewing for a minimum period of 6 months were included.

Exclusion criteria

- Individuals who were diagnosed with periodontitis
- Individuals with systemic diseases/conditions such as diabetes, renal failure, liver cirrhosis and bone disorders such as rickets, obstructive jaundice and hyperparathyroidism
- Individuals taking medication that could alter salivary characteristics.

The individuals were explained about the purpose of the study, and informed consent was obtained. A volume of 3 ml of unstimulated saliva was collected from all individuals by spitting method.[5] The individuals were instructed not to take food for 2 h prior to saliva collection. They were asked to rinse their mouth with water and 10 min later, they were advised to sit upright with head slightly tilted forward to collect saliva in the floor of the mouth and then spit into a sample container. The samples were then centrifuged at 3000 rpm for 15 min,[6] and the supernatant saliva was obtained. 20 μl of the supernatant was mixed with 1000 μl of ALP reagent (Alkaline Phosphatase (ALP)-AMP kit, Biosystems S.A., Barcelona) for the estimation of S-ALP levels in an automatic analyzer (BA 400, Biosystems). S-ALP concentrations were expressed in terms of IU/L.

Principle

The level of ALP was measured by the kinetic photometric test according to the International Federation of Clinical Chemistry and Laboratory Medicine based on the principle that ALP converts p-nitrophenyl phosphate into phosphate and p-nitrophenol, which was measured at 405 nm.[7] Data obtained were subjected to statistical analysis.

RESULTS

The mean values for S-ALP were found to be about 18.00 IU/L (standard deviation [SD] 13.376) for Group I (range from 7 to 50 IU/L), 7.50 IU/L (SD 4.353) for Group II (range from 2 to 14 IU/L), 4.60 IU/L (SD 2.011) for Group III (range from 1 to 8 IU/L) and 64.90 IU/L (SD 51.707) for Group IV (range from 10 to 146 IU/L) [Figure 1]. Comparison of S-ALP between the groups showed a statistically significant difference ($P < 0.001$) using Kruskal–Wallis' ANOVA [Table 1].

To know if there is any difference in S-ALP levels between tobacco users and nonusers (Groups I and II and Groups I and III) and among the different forms of tobacco users (Groups II and III), the mean S-ALP levels were compared using Mann–Whitney U-test. No significant difference was found in the mean S-ALP levels between tobacco users and nonusers. Similarly, the difference in the mean S-ALP levels between smokers and tobacco chewers was also statistically insignificant ($P > 0.05$) [Table 2]. This shows that there is not much difference in S-ALP levels in the nonlesional group irrespective of the tobacco habit.

To know if there is any difference in the mean S-ALP levels in individuals with lesion (Group IV) and without lesion (Groups I, II and III), comparison of mean S-ALP levels was made using Mann–Whitney U-test. S-ALP levels in lesional group were found to be statistically significantly higher ($P < 0.001$) than those without lesions [Table 2]. This shows that S-ALP levels are significantly increased in OPMD irrespective of the tobacco habit.

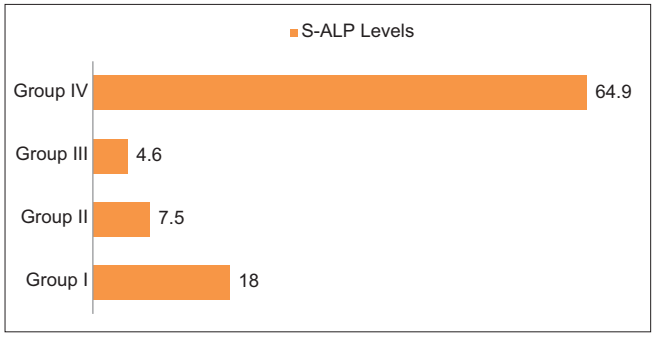

Figure 1: Bar diagram showing mean salivary alkaline phosphatase levels between the groups

Table 1: Comparison of salivary alkaline phosphatase between the groups

Group	*n*	Mean	SD	*P*
I	12	18.00	13.376	<0.001*
II	10	7.50	4.353	
III	10	4.60	2.011	
IV	10	64.90	51.707	
Total	42	23.48	34.877	

*Statistically significant using Kruskal-Wallis's ANOVA ($P<0.001$).
SD: Standard deviation

DISCUSSION

Tobacco is a major etiological factor in the development of most of the OPMDs. Unfortunately, India is the second largest consumer of tobacco[8] in the world after China, in both smoking and smokeless forms. The smokeless forms include betel quid chewing and consumption of mishri, khaini, gutka, snuff and also an ingredient of pan masala. The smoking forms of tobacco are cigarettes, bidis, hooka, hookli, chhutta, dhumti and chillum.[9] The main carcinogens in tobacco products such as tobacco-specific nitrosamines, aldehydes, phenols, nitro compounds and polycyclic aromatic hydrocarbons[10] induce mutations in the genetic material of oral epithelial cells that may lead to the development of OSCC which is most often preceded by OPMD.

Some of the most commonly encountered OPMDs associated with tobacco habits include leukoplakia, oral submucous fibrosis, erythroplakia and palatal lesions among reverse smokers.[11] The potential for malignant transformation among OPMDs may vary from <1% to as high as 36%.[12] Early detection and intervention at the OPMD level will greatly reduce the morbidity associated with the malignancy. ALP is recognized as an important marker of induction of tumor cell differentiation,[13] and its levels in saliva are known to increase in squamous cell carcinoma.[14] However, its salivary levels in OPMD have been less explored.

ALP belongs to hydrolase group of enzymes which are biocatalysts synthesized in living cells. ALP functions by catalyzing the hydrolysis of monoesters of phosphoric acid and also transphosphorylation reaction in the presence of large concentrations of phosphate acceptors.[15] The normal levels of ALP in saliva range between 5.50 and 12.58 IU/L.[6] The source of this enzyme in the oral cavity includes neutrophils, bacteria and oral epithelial cells.[14,16]

In the present study, the S-ALP levels were estimated among tobacco users (Groups II and III), nonusers (Group I) and in individuals with OPMD (Group IV). The mean S-ALP obtained was 18.00 IU/L for normal individuals without

Table 2: Group wise comparison of salivary alkaline phosphatase

Group	*n*	Mean	SD	Group wise comparison - mean difference					
				Group I versus II	Group I versus III	Group II versus III	Group I versus IV	Group II versus IV	Group III versus IV
I	12	18.00	13.376	10.500#	13.400#	2.900#	46.900*	57.400*	60.300*
II	10	7.50	4.353						
III	10	4.60	2.011						
IV	10	64.90	51.707						
Total	42	23.48	34.877						

*Statistically significant, #Statistically not significant using Mann-Whitney U-test. SD: Standard deviation

tobacco usage. This is similar to the values obtained by Prakash *et al*.[6] and Dhivyalakshmi and Maheswari.[17] The mean S-ALP level in smokers without lesion was 4.60 IU/L, which is similar to the results obtained by Kibayashi *et al*.[18] The mean S-ALP level in tobacco chewers without any lesion was 7.50 IU/L.

In our study, the mean S-ALP level was lower in tobacco users than controls though the difference was statistically insignificant. The lower value observed could be because of the deleterious effect of smoking and chewing tobacco on the oral environment. Tobacco lowers the salivary pH which in turn can affect salivary enzyme activity including S-ALP.[19] In addition, the physical, mechanical and chemical irritation caused by tobacco leads to keratosis,[19] which again may reduce the release of ALP in saliva. However, this can be validated by performing studies with increased sample size.

To know if S-ALP levels could be used as a biomarker for the early detection of OPMD, we compared the S-ALP levels in individuals with OPMD and those without lesion irrespective of their tobacco usage habits. We found that S-ALP levels were significantly higher in individuals with OPMD. Similar increase in S-AL*P* values was observed by Prakash *et al*.[6] and Dhivyalakshmi and Maheswari.[17] in individuals with leukoplakia. This shows that S-ALP in saliva can potentially reflect the changes associated with OPMD.

The increased S-ALP levels observed in OPMD cases could be secondary to the increase in the oxidative stress[20,21] associated with the lesion. The rise in reactive oxygen species[6,20] induces cellular damage,[21,22] which leads to increased release of ALP in saliva. The increased rate of cellular turnover in OPMD,[23] either as a compensatory mechanism or due to genetic mutation, can also lead to increase in ALP production by epithelial cells. The increased inflammatory reaction[24] seen in association with OPMD could also be another contributing factor for the high levels of S-ALP observed.

The current study is an initiative to detect the early signals in the malignant transformation of OPMD in a simple, economic and noninvasive manner by monitoring S-ALP enzyme. The results obtained in our study are promising, and can be expanded further with a larger sample size for a better understanding of the association between the S-ALP levels and OPMD.

CONCLUSION

We compared the S-ALP levels in individuals with and without the habit of using smoking/smokeless tobacco and in patients with OPMD to determine the potential role of S-ALP enzyme as a biomarker in OPMD. The result of the current research suggests that S-ALP could be used as a reliable noninvasive biomarker to monitor OPMD. This study is yet another step to embrace salivary diagnostics in future.

Financial support and sponsorship

This study was financially supported by the college's internal fund.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Sarode SC, Sarode GS, Tupkari JV. Oral potentially malignant disorders: A proposal for terminology and definition with review of literature. J Oral Maxillofac Pathol 2014;18:S77-80.
2. Tandon P, Dadhich A, Saluja H, Bawane S, Sachdeva S. The prevalence of squamous cell carcinoma in different sites of oral cavity at our rural health care centre in Loni, Maharashtra – A retrospective 10-year study. Contemp Oncol (Pozn) 2017;21:178-83.
3. Shetty SR, Al-Bayati SA, Hamed MS, Abdemagyd HA. Salivary alkaline phosphatase and oral health: A review. Italian Journal of Dental Medicine 2017;2:55-8.
4. Acharya S, Kale J, Rai P, Anehosur V, Hallikeri K. Serum alkaline phosphatase in oral squamous cell carcinoma and its association with clinicopathological characteristics. South Asian J Cancer 2017;6:125-8.
5. Muddugangadhar BC, Sangur R, Rudraprasad IV, Nandeeshwar DB, Kumar BH. A clinical study to compare between resting and stimulated whole salivary flow rate and pH before and after complete denture placement in different age groups. J Indian Prosthodont Soc 2015;15:356-66.
6. Prakash AR, Indupuru K, Sreenath G, Kanth MR, Reddy AV, Indira Y. Salivary alkaline phosphatase levels speak about association of smoking, diabetes and potentially malignant diseases??? J Oral Maxillofac Pathol 2016;20:66-70.
7. Jazaeri M, Malekzadeh H, Abdolsamadi H, Rezaei-Soufi L, Samami M. Relationship between salivary alkaline phosphatase enzyme activity and the concentrations of salivary calcium and phosphate ions. Cell J 2015;17:159-62.
8. Mishra GA, Pimple SA, Shastri SS. An overview of the tobacco problem in India. Indian J Med Paediatr Oncol 2012;33:139-45.
9. Chadda R, Sengupta S. Tobacco use by Indian adolescents. Tob Induc Dis 2002;1:111-9.
10. Xue J, Yang S, Seng S. Mechanisms of cancer induction by tobacco-specific NNK and NNN. Cancers (Basel) 2014;6:1138-56.
11. Warnakulasuriya S. Clinical features and presentation of oral potentially malignant disorders. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:582-90.
12. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: Risk of progression to malignancy. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:612-27.
13. Bhattacharjee A, Giri S, Roy M, Chakraborty A. Correlation of serum lactate dehydrogenase and alkaline phosphatase in different histological grades of head and neck squamous cell carcinoma and premalignant lesions. J Cancer Res Ther 2018;14:934-40.
14. Abdullah BH, Alaaraji SB, Merza KS. Comparative study on lactate dehydrogenase, alkaline phosphatase and immunoglobulins in serum and saliva of acute leukemia and oral squamous cell carcinoma patients.

Iraqi J Sci 2010;51:262-70.
15. Millán JL. Alkaline phosphatases: Structure, substrate specificity and functional relatedness to other members of a large superfamily of enzymes. Purinergic Signal 2006;2:335-41.
16. Sanikop S, Patil S, Agrawal P. Gingival crevicular fluid alkaline phosphatase as a potential diagnostic marker of periodontal disease. J Indian Soc Periodontol 2012;16:513-8.
17. Dhivyalakshmi M, Maheswari TU. Expression of salivary biomarkers–Alkaline phosphatase & lactate dehydrogenase in oral leukoplakia. Int J Chemtech Res 2014;6:3014-8.
18. Kibayashi M, Tanaka M, Nishida N, Kuboniwa M, Kataoka K, Nagata H, *et al*. Longitudinal study of the association between smoking as a periodontitis risk and salivary biomarkers related to periodontitis. J Periodontol 2007;78:859-67.
19. Ahmadi-Motamayel F, Falsafi P, Goodarzi MT, Poorolajal J. Comparison of salivary pH, buffering capacity and alkaline phosphatase in smokers and healthy non-smokers: Retrospective cohort study. Sultan Qaboos Univ Med J 2016;16:e317-21.
20. Khanna A, Guo M, Mehra M, Royal W 3rd. Inflammation and oxidative stress induced by cigarette smoke in Lewis rat brains. J Neuroimmunol 2013;254:69-75.
21. Bagchi M, Balmoori J, Bagchi D, Ray SD, Kuszynski C, Stohs SJ. Smokeless tobacco, oxidative stress, apoptosis, and antioxidants in human oral keratinocytes. Free Radic Biol Med 1999;26:992-1000.
22. Aoshiba K, Nagai A. Oxidative stress, cell death, and other damage to alveolar epithelial cells induced by cigarette smoke. Tob Induc Dis 2003;1:219-26.
23. Saxena S. Assessment of Ki-67 in healthy, premalignant, and malignant lesions of the oral mucosa and its predictive role in disease progression. Int J Oral Med Sci 2010;9:67-74.
24. Mashhadiabbas F, Fayazi-Boroujeni M. Correlation of vascularization and inflammation with severity of oral leukoplakia. Iran J Pathol 2017;12:225-30.