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

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Abstract---Background: Although elevated salivary alkaline phosphatase (S-ALP) levels have been seen in oral squamous cell carcinoma, the status of S-ALP in cigarette users and those with oral

potentially malignant disorders (OPMDs) is less well understood. The study's goals and objectives were to assess and compare S-ALP levels in tobacco users, nonusers, and people with OPMD. **Materials and Methods:** The study included 150 people who were divided into four groups: those who did not use tobacco, tobacco smokers, those who are in habit of tobacco chewing, and those who are in the habit of tobacco chewing/smoking and had a lesion. unstimulated saliva (5 mL) was collected and centrifuged for 15 minutes at 3000 rpm, and the supernatant was separated. In an automatic analyzer, S-ALP was calculated in the supernatant using the kinetic photometric method. **Results:** The information gathered was subjected to statistical analysis. S-ALP levels were 17.90 IU/L in healthy people who didn't smoke, 4.58 IU/L in smokers who didn't have a lesion, and 7.52 IU/L in tobacco chewers who didn't have a lesion, and 64.92 IU/L in people with OPMD. Using Kruskal-Wallis' ANOVA, the mean difference between the groups was statistically significant (P 0.001). Using the Mann-Whitney U-test, S-ALP levels in people with OPMD were statistically substantially greater (P 0.001) than those without lesions, with or without a tobacco use habit. **Conclusion:** We believe that S-ALP could be a useful noninvasive biomarker for OPMD monitoring.

Keywords---biomarkers, saliva, salivary alkaline phosphatase, smokeless tobacco, smoking.

Introduction

Oral potentially malignant disorders (OPMDs), a term coined by the World Health Organization in 2007¹ to describe premalignant lesions and syndromes, have been linked to a high risk of developing into oral cancer. In the Indian population, oral squamous cell carcinoma (OSCC) accounts for more than 30% of all cancers.² although several etiologic variables have been proposed, tobacco use is a well-known cause of OPMD and OSCC. Saliva, which is an easily accessible oral fluid, has gained acceptability as a diagnostic medium in numerous health disorders in recent years.³ It can detect early mucosal changes in tobacco users and people with OPMD since it is in direct contact with the lesion. Although only a few studies have been published on the use of alkaline phosphatase (ALP) enzyme levels as a biomarker in OSCC patients' serum and saliva, more research is needed on OPMD.⁴ As a result, we conducted this study to investigate salivary ALP (S-ALP) levels in tobacco users and people with OPMD to see if it may be used as a biomarker.

Aim and Objectives

The goal of this study was to determine the levels of S-ALP in tobacco users, nonusers, and people with OPMDs. The following were the study's key goals:

- To determine the amounts of S-ALP in smokers, chewing tobacco users, nonsmokers, and people with OPMD.

- To compare the levels of S-ALP in smokers, chewing tobacco users, non-smokers, and people with OPMD.

Materials and Method

The participants in the study ranged in age from 20 to 70 years. There were a total of 150 participants and they were divided into four groups:

- Individuals who did not smoke or chew tobacco and did not have any lesions on intraoral inspection (n = 39) were assigned to Group I.
- Individuals who consume tobacco but do not have any lesions on intraoral inspection (n = 37) were assigned to Group II.
- Individuals with a smoking habit but no intraoral lesion (n = 37) were assigned to Group III.
- Individuals with a lesion on intraoral inspection and a habit of smoking/chewing tobacco (n = 37) were assigned to Group IV.

Criteria for inclusion

Individuals who had the habit of smoking/chewing tobacco for a minimum of 6 months (1st January 2021 to 30th June 2021) were included in Group II, Group III, and Group IV.

Criteria for exclusion

- People who have been diagnosed with periodontitis
- Individuals suffering from diabetes, renal failure, liver cirrhosis, and bone problems such as rickets, obstructive jaundice, and hyperparathyroidism etc. or any other systemic disorders.
- People who are taking medications that may cause changes in salivary composition.

The participants were informed about the study's purpose, and their informed consent was obtained. The spitting method was used to collect 3 cc of unstimulated saliva from each participant.⁵ Before saliva collection, the participants were told not to eat for two hours. They were instructed to clean their mouths with water and then sit upright with their heads slightly leaned forward for 10 minutes to collect saliva from the floor of their mouth and spit into a sample container. The samples were then centrifuged at 3000 rpm for 15 minutes⁶ to get the supernatant saliva. For the estimation of S-ALP levels in an automatic analyzer, 20 µl of supernatant was combined with 1000 µl of ALP reagent (Alkaline Phosphatase (ALP)-AMP kit, Biosystems S.A., Barcelona) (BA 400, Biosystems). S-ALP concentrations were measured in IU/L.

Principle

The level of ALP was determined using the International Federation of Clinical Chemistry and Laboratory Medicine's kinetic photometric technique, which is based on the idea that ALP transforms p-nitrophenyl phosphate into phosphate and p-nitrophenol, which was detected at 405 nm.⁷ the information gathered, was

subjected to statistical analysis. The mean difference between the groups were compared using Kruskal–Wallis' ANOVA. Mann–Whitney U-test was used to do intergroup comparison. The statistical significance was considered if the values were less than 0.001.

Results

S-ALP mean values were discovered to be around 17.90 IU/L (SD 13.386) for Group I (range 6 to 45 IU/L), 7.52 IU/L (SD 4.355) for Group II (range 3 to 15 IU/L), 4.58 IU/L (SD 2.014) for Group III (range 1 to 9 IU/L), and 64.92 IU/L (SD 51.717) for Group IV (range 11 to 145 IU/L). Using Kruskal–Wallis' ANOVA, the comparison of S-ALP between the groups revealed a statistically significant difference ($P < 0.001$) [Table 1].

Table 1
Comparison of salivary alkaline phosphatase between the groups

Group	n	mean	SD	p
I	39	17.90	13.386	<0.001*
II	37	7.52	4.355	
III	37	4.58	2.014	
IV	37	64.92	51.717	
Total	150	23.46	34.867	

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

The mean S-ALP levels were compared using the Mann–Whitney U-test to see if there was any difference in S-ALP levels between tobacco users and nonusers (Groups I and II and Groups I and III) and between the different forms of tobacco users (Groups II and III). There was no discernible difference in mean S-ALP levels between cigarette users and nonusers. Similarly, there was no statistically significant difference in mean S-ALP levels between smokers and tobacco chewers ($P > 0.05$) [Table 2]. This demonstrates that, regardless of cigarette use, there is little variation in S-ALP levels in the non-lesional group. The Mann–Whitney U-test was used to see if there was any difference in mean S-ALP levels between persons with a lesion (Group IV) and those without a lesion (Groups I, II, and III). S-ALP levels were observed to be statistically substantially higher ($P < 0.001$) in the lesional group than in the control group [Table 2]. This reveals that, regardless of cigarette use, S-ALP levels are considerably higher in people with OPMD.

Table 2
Group-wise comparison of salivary alkaline phosphatase

Group	n	mean	SD	Groupwise comparison of mean difference					
				Group I vs. II	Group I vs. III	Group I vs. IV	Group II vs. III	Group II vs. IV	Group III vs. IV
I	39	17.90	13.386	10.600	13.300	46.800	2.800#	57.500	60.400

II	37	7.52	4.355	#	#	*		*	*
III	37	4.58	2.014						
IV	37	64.92	51.717						
Total	150	23.46	34.867						

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

Discussion

Tobacco use is a major contributor to the development of most OPMDs. Unfortunately, after China, India is the world's second-largest consumer of tobacco⁸, in both smoking and non-smoking forms. Chewing betel quid and consuming mishri, khaini, gutka, snuff, and as an ingredient in the pan masala are among the smokeless forms. Cigarettes, bidis, hookah, hookli, chhutta, dhumti, and chillum are all tobacco smoking methods.⁹ Tobacco-specific nitrosamines, aldehydes, phenols, nitro compounds, and polycyclic aromatic hydrocarbons¹⁰ create changes in the genetic material of oral epithelial cells, which may contribute to the development of OSCC, which is frequently preceded by OPMD.

Leukoplakia, oral submucous fibrosis, erythroplakia, and palatal lesions are some of the most prevalent OPMDs associated with tobacco use among reverse smokers.¹¹ the likelihood of malignant transformation in OPMDs might range from 1% to 36%.¹² The morbidity associated with cancer will be considerably reduced if it is detected and treated early at the OPMD level. ALP is a well-known marker of tumor cell differentiation induction¹³, and its levels in saliva are known to rise in squamous cell carcinoma.¹⁴ Salivary levels in OPMD, on the other hand, have received little attention. ALP is a member of the hydrolase family of enzymes, which are biocatalysts produced in living cells. ALP catalyzes the hydrolysis of phosphoric acid monoesters as well as the transphosphorylation process in the presence of high amounts of phosphate acceptors.¹⁵ ALP levels in saliva should be between 5.50 and 12.58 IU/L.⁶ Neutrophils, bacteria, and oral epithelial cells are all sources of this enzyme in the oral cavity.¹⁴

The levels of S-ALP were measured in tobacco users (Groups II and III), nonusers (Group I), and people with OPMD in this study (Group IV). For healthy people who did not smoke, the average S-ALP level was 17.90 IU/L. Prakash et al.⁶ and Dhivyalakshmi and Maheswari came up with similar results.¹⁶ The average S-ALP level among smokers without a lesion was 4.58 IU/L, which is similar to Kibayashi et al findings.¹⁷ In tobacco chewers who had no lesion, the mean S-ALP level was 7.52 IU/L. The mean S-ALP level in tobacco users was lower compared to controls in our study, while the difference was statistically insignificant. Tobacco also reduces salivary pH, which has an impact on salivary enzyme activity, particularly S-ALP.¹⁸ Furthermore, smoke causes physical, mechanical, and chemical irritation, which leads to keratosis¹⁸, which may limit the release of ALP in saliva.

To see if S-ALP levels may be utilized as a biomarker for early identification of OPMD, researchers examined S-ALP levels in people with OPMD with people without the disease, regardless of whether or not they smoked tobacco. S-ALP

levels were observed to be considerably greater in people with OPMD. Prakash et al.⁶ and Dhivyalakshmi and Maheswari.¹⁶ found a similar increase in S-ALP values in people with leukoplakia. This indicates that S-ALP levels in saliva may reflect OPMD-related alterations. Increased S-ALP levels in OPMD cases could be a result of the increased oxidative stress associated with the disease.^{19,20} Increased production of ALP in the saliva is caused by an increase in reactive oxygen species⁶, which causes cellular damage.²⁰ Increased cellular turnover in OPMD can lead to an increase in ALP synthesis by epithelial cells, either as a compensatory mechanism or due to genetic mutation.²¹ The enhanced inflammatory response associated with OPMD could be another reason contributing to the high amounts of S-ALP was seen.²² The current work is an effort to detect early signs of OPMD malignant transformation in a straightforward, cost-effective, and noninvasive method by monitoring the S-ALP enzyme. The findings of our study are encouraging, and they can be expanded with a bigger sample size to gain a better knowledge of the relationship between S-ALP levels and OPMD.

Conclusion

To assess the possible role of the S-ALP enzyme as a biomarker in OPMD, we compared S-ALP levels in persons with and without the habit of using smoking/chewing tobacco and in patients with OPMD. The current study findings show that S-ALP could be employed as a viable noninvasive biomarker to monitor OPMD. This study is just another step toward the future acceptance of salivary diagnostics.

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