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

Dr Arvind Jain
Assistant professor, Department of Oral pathology, Maitri college of dentistry and research, Chhattisgarh
Corresponding author email: arvind@jaindental.com

Dr Deepika Jain
Assistant professor, Department of Pedodontics and preventive dentistry, Maitri college of dentistry and research, Chhattisgarh
Email: arvind@jaindental.com

Dr Sohil Jain
Assistant professor, Department of Periodontology, Maitri college of dentistry and research, Chhattisgarh
Email: arvind@jaindental.com

Dr Sumit Jaiswal
Assistant professor, Department of Oral pathology, Maitri college of dentistry and research, Chhattisgarh
Email: sumit1331988@gmail.com

Dr Ravi Kumar Goyal
Private Practitioner, Orthodontics
Email: ravigoyal@gmail.com

***Abstract*---**Background: Saliva, a widely obtainable oral fluid, has gained acceptability as a screening medium in numerous health disorders in recent years. It has the capacity to discern initial epithelial alterations in tobacco abusers and people with OPMD since it is in direct contact with the lesion. Although few reports have been conducted on the use of concentration of alkaline phosphatase (ALP) enzyme in serum as well as saliva of OSCC patients as a biomarker, more research is needed in OPMD.Aims and Objectives: The study's major goals were to measure S-ALP levels in smokers, nonsmokers, and people with OPMD. and to compare S ALP levels in smokers,

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nonsmokers, and people with OPMD.Materials and Methods: The 84 participants in the study ranged in age from 18 to 75 years old, and they were divided into four groups:Category One – People who don't smoke or chew tobacco and don't have any lesions on intraoral inspection (n = 24). Category two – People who use tobacco but don't have any lesions on intraoral inspection (n = 20).Category three (n = 20) — Smokers who did not have any lesions on intraoral inspection.Category four (n = 20): Individuals having an intraoral lesion and a habit of smoking or chewing tobacco. The level of ALP was determined using the Worldwide Association of Clinical Chemistry and Laboratory Medicine's kinetic photometric technique, which is based on the idea that ALP transforms p nitrophenyl phosphate compound into phosphate and p nitrophenol compound, which was detected at 405 nm. The information gathered was analyzed by using statistical package. Results: S-ALP mean values were discovered to be around 19.11 14.487 IU/L in category one, 8.61 5.464 IU/L in category two, 5.71 3.122 IU/L in category three, and 65.01 52.818 IU/L in category four. There was no discernible variation in mean S-ALP levels between tobacco users and nonusers.Similarly, there was no statistically significant difference in mean S ALP concentration between tobacco smokers & tobacco chewers (P > 0.05) [Table 2]. This demonstrates that, regardless of cigarette use, there is little variation in S ALP concentrations in the non-lesional group. Conclusion: The current study's findings show that S ALP could be employed as a viable noninvasive biomarker for OPMD monitoring. This research is yet another step toward the adoption of salivary diagnostics in the future.

***Keywords*---**salivary alkaline phosphatase, tobaaco users, OMPDs.

Introduction

Oral potentially malignant disorders considered as OPMDs is a term coined by the World Health Organization in 2007 to describe premalignant lesions and syndromes, have been linked to a high risk of developing into squamous cell carcinoma of oral cavity (OSCC). In the Indian population, OSCC accounts for more than 30% of all cancers. Despite the fact that several etiologic variables have indeed been hypothesized, tobacco use is a well-known cause of premalignant disorders and cancer of oral cavity.[1] Despite the fact that tissue biopsy as well as routine histopathology are the gold standard procedures for diagnosing lesions, biopsy is an traumatic method, and the lesion is usually progressed at the time of diagnosis. As a result, having a proven noninvasive option for early identification and analysis of OPMD in at-risk individuals will dramatically minimise OSCC morbidity.[2]

Saliva, a widely obtainable oral fluid, has gained acceptability as a screening medium in numerous health disorders in recent years. It has the capacity to discern initial epithelial alterations in tobacco abusers and people with OPMD since it is in direct contact with the lesion. Although few reports have been

conducted on the use of concentration of alkaline phosphatase (ALP) enzyme in serum as well as saliva of OSCC patients as a biomarker, more research is needed in OPMD. As a result, we conducted this study to investigate levels of salivary ALP generally considered as S-ALP in tobacco abusers and people with OPMD to see if it may be used as a biomarker.[3,4] The goal of this study was to see how much S ALP was present in tobacco users, nonusers, and those with OPMDs. The study's major goals were to measure S ALP levels in smokers, nonsmokers, and people with OPMD. • To compare S ALP levels in smokers, nonsmokers, and people with OPMD.

Materials and Methods

The 84 participants in the study ranged in age from 18 to 75 years old, and they were divided into four groups:

- Category One – People who don't smoke or chew tobacco and don't have any lesions on intraoral inspection (n = 24).
- Category two – People who use tobacco but don't have any lesions on intraoral inspection (n = 20).
- Category three (n = 20) — Smokers who did not have any lesions on intraoral inspection.
- Category four (n = 20): Individuals having an intraoral lesion and a habit of smoking or chewing tobacco.

Criteria for inclusion

Individuals who had smoked/tobacco chewed for a duration of six months were included in category two, category three, and category IV.

Criteria for exclusion

- People with periodontitis
- People with medical disorders such as diabetes mellitus, kidney damage, cirrhosis of the liver, and musculoskeletal diseases like rickets, obstructive jaundice, and hyperparathyroidism
- People who are taking medications that may cause changes in salivary characteristics.

The participants were informed about the study's purpose, and their informed written consent was obtained. The spitting method was used to collect 3 cc of un-stimulated saliva from each participant. Prior to saliva collection, the participants were told not to eat for two hours. They were instructed to rinse their mouths with water and then sit erect with their heads slightly leaned forward for 10 minutes to gather saliva in the mouth's floor and afterwards spit it into a glass beaker. The saliva specimens were then centrifuged for 15 minutes at 3000 rpm to get the supernatant saliva. For the determination of S ALP levels in an automatic analyzer, 20 l of the supernatant was combined with 1000 l of ALP reagent (Alkaline Phosphatase (ALP)-AMP kit, Barcelona). The concentrations of S ALP were measured in IU/L.

Principle

The level of ALP was determined using the Worldwide Association of Clinical Chemistry and Laboratory Medicine's kinetic photometric technique, which is based on the idea that ALP transforms p nitrophenyl phosphate compound into phosphate and p nitrophenol compound, which was detected at 405 nm. The information gathered was analyzed by using statistical package.

Results

S-ALP mean values were discovered to be around 19.11 14.487 IU/L in category one, 8.61 5.464 IU/L in category two, 5.71 3.122 IU/L in category three, and 65.01 52.818 IU/L in category four. The Kruskal–Wallis test found a significant difference statistically (P 0.001) in S ALP between the groups. 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 (categories one and three) and between the different types of tobacco users (categories two and three). There was no discernible variation in mean S ALP levels between tobacco users and nonusers.(Table 1).

Similarly, there was no statistically significant difference in mean S ALP concentration between tobacco smokers & tobacco chewers (P > 0.05) [Table 2]. This demonstrates that, regardless of cigarette use, there is little variation in S ALP concentrations 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 those having lesions (category four) and people without lesions (categories one, two, and three). 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 concentrations are considerably higher in OPMD patients.

Table 1: Category wise comparison of salivary alkaline phosphatase

Category	n values	Mean values	SD valaues	P value
One	24	19.11	14.487	<0.001*
Two	20	8.61	5.464	
Three	20	5.71	3.122	
Four	20	65.01	52.818	
Total	84	24.59	35.988	

Table 2: Category wise comparison - mean difference

Group I versus II	10.611#
Group I versus III	13.511#
Group II versus III	2.911#
Group I versus IV	46.945*

Group II versus IV	57.437*
Group III versus IV	60.354*

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

Discussion

Tobacco use is a major driving force for the occurrence of most OPMDs. Tragically, after China, India is the world's second-largest user of tobacco, in both smoking forms and non-smoking forms. Chewing betel quid and consuming mishri products, khaini products, gutka products, snuff, and as an ingredient in pan masala are among the smokeless forms. Cigarette products, bidis products, hooka, hookli, Cohutta, dhumti, and chillum are all tobacco smoking methods. Tobacco-specific nitrosamines compounds, aldehyde compounds, phenols compounds, nitro compounds, and polycyclic aromatic hydrocarbons compounds cause changes in the genetic content of oral epithelial cells, which can lead to OSCC, which is frequently preceded by OPMD.[5,6]

Leukoplakia, oral submucous fibrosis, erythroplakia, and palatal lesions are some of the most prevalent OPMDs associated with tobacco use among reverse smokers. The likelihood of malignant transformation in OPMDs might range from 1% to 36%. The incidence of death associated with cancer will be considerably reduced if it is detected and treated early at the OPMD level. The mean S ALP level among smokers without a lesion in the oral cavity was 5.71 IU/L, which is consistent with Kibayashi et al findings. In tobacco chewers who had no lesion, the mean S ALP concentration was 8.61 IU/L. The mean S ALP level in cigarette smokers was lower than in controls in our study, albeit the variation was statistically insignificant.[7,8]

ALP is a member of the hydrolase family of enzymes, which are biocatalysts produced in living cells. ALP works by catalysing the hydrolysis of phosphoric acid monoesters also in the presence of a transphosphorylation reaction phosphate acceptors in high concentrations. The ALP levels in saliva should be between 5.50 and12.58 IU/L (International Units per Liter). The mouth cavity is the source of this enzyme. Neutrophils, bacteria, and oral epithelial cells are all included.[9]

Because of the harmful effects of smoking tobacco and chewing tobacco on the oral tissues, the lower value found could be due to this. Tobacco reduces salivary pH, which has an impact on salivary enzyme activity, especially S ALP. Furthermore, smoke causes physical, mechanical, and chemical irritation, which leads to keratosis, which may limit the secretion of ALP in saliva. This, however, can be confirmed by conducting research with larger sample size. To see if S -ALP levels may be utilised as a biomarker for early identification of OPMD, researchers examined S ALP levels in those individuals with OPMD and those individuals without a lesion, regardless of whether or not they smoked. S ALP concentrations were determined to be considerably greater in those with OPMD.[10,11]

Prakash et al. as well as Dhivyalakshmi and Maheswari. found a similar increase in S ALP concentrations in people with leukoplakia. This indicates that S ALP levels in saliva may represent OPMD-related alterations. Increased S ALP levels in OPMD patients could be a result of the increased oxidative stress linked with this condition. Increased production of ALP in the saliva is caused by an increase in reactive oxygen species, which causes cellular damage. Increased cellular turnover in OPMD can lead to an increase in ALP synthesis by epithelial cells, either as a reactive response or due to genetic mutation.[12] The enhanced inflammatory response seen could be another reason leading to the elevated levels of S ALP found.[13] The current study aims to use S ALP enzyme monitoring to detect early signs of OPMD's malignant change in a straightforward, cost-effective, and noninvasive method. The findings of our study are encouraging, and they can be further expanded with a bigger sample size to gain an insight into the relationships between S ALP concentrations and OPMD

Conclusion

The current study's findings show that S ALP could be employed as a viable noninvasive biomarker for OPMD monitoring. This research is yet another step toward the adoption of salivary diagnostics in the future.

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