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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

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**Abstract**--The aim of this study evaluate the salivary alkaline phosphatase levels in tobacco users to determine its role as a biomarker in oral potentially malignant disorders. This prospective observational study comprised 60 individuals aged between 18 and 70 years who were categorized into four groups. For Group I, Group II, Group III and Group IV, individuals with the habit of smoking/tobacco chewing for a minimum period of 6 months were included in this study. The mean values for S-ALP were found to be about 19.00 IU/L (SD 12.37) for Group I (range from 7 to 50 IU/L), 8.50 IU/L (SD 3.35) for Group II (range from 2 to 14 IU/L), 5.60 IU/L (SD 1.01) for Group III (range from 1 to 8 IU/L) and 65.90 IU/L (SD 50.70) for Group IV (range from 10 to 146 IU/L). Comparison of S-ALP between the groups showed a statistically significant difference ( $P < 0.001$ ) using Kruskal–Wallis' ANOVA. 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 ( $P > 0.05$ ). 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.

**Keywords**---salivary alkaline phosphatase, tobacco, malignant disorders.

## Introduction

Detection of disease in its early stages is a key to its prognostic outcome. Early detection tools need to be easy to obtain and non-invasive, which makes salivary diagnostics as one of the suitable alternatives to blood. There are multiple advantages when saliva is used as a diagnostic tool compared to serum or tissues. In fact, its non-invasive method of collection, smaller sample fraction, good patient compliance, cost effectiveness, easy storage and transportation, greater sensitivity, and correlation with levels in blood are the advantages of salivary diagnostics.<sup>1</sup> Moreover, owing to emerging new technologies, salivary biomarkers have been developed for a wide range of medical conditions such as malignancies, autoimmune disorders, infections and metabolic diseases.<sup>2</sup> Among the salivary biomarkers alkaline phosphatases have been of prime interest especially from the dental aspects. Saliva is an oral fluid that has been used as a diagnostic tool in medicine and dentistry. The source of the specimen that can be used for salivary markers are whole saliva, gingival crevicular fluid (GCF) and plaque. Among these, enzymes released from the host can be easily obtained within the oral cavity either from GCF or from the whole saliva.<sup>3</sup> Several enzymes evaluated for the early diagnosis of periodontal disease are lactate dehydrogenase, alkaline phosphatase (ALP), acid phosphatase, aspartate aminotransferase and alanine aminotransferase. Sampling technique for GCF collection is a time-consuming process and is a difficult procedure.<sup>4</sup>

Oral potentially malignant disorders (OPMDs), a terminology suggested by the World Health Organization in 2007 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. Although many etiologic factors have been proposed, tobacco product is a well-established etiology for the development of OPMD and OSCC.<sup>5</sup> Cigarette smoking is known to be one of the major causes of various health disorders. These toxic components can predispose to different systemic disorders, such as cardiac diseases, cancers, precancerous lesions and pulmonary disorders. Saliva is the first body fluid to encounter cigarette smoke. The salivary antioxidant system plays a very important role in the anti-carcinogenic capacity of saliva and includes various enzymes and molecules, such as uric acid, peroxidase system and phosphatases.<sup>6</sup>

## Material and Methods

The study population comprised 60 individuals aged between 18 and 70 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 ( $n = 15$ )
- Group II – Individuals with the habit of chewing tobacco and without any lesion on intraoral examination ( $n = 15$ )

- Group III – Individuals with the habit of smoking and without any lesion on intraoral examination ( $n = 15$ )
- Group IV – Individuals with lesion on intraoral examination with the habit of smoking/chewing tobacco ( $n = 15$ ).

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 in this study. 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 and Individuals taking medication that could alter salivary characteristics were excluded from the study.

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.<sup>7</sup> 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,<sup>8</sup> and the supernatant saliva was obtained. 20  $\mu$ l of the supernatant was mixed with 1000 $\mu$ l 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. 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.<sup>9</sup>

## Results

The mean values for S-ALP were found to be about 19.00 IU/L (standard deviation [SD] 12.37) for Group I (range from 7 to 50 IU/L), 8.50 IU/L (SD 3.35) for Group II (range from 2 to 14 IU/L), 5.60 IU/L (SD 1.01) for Group III (range from 1 to 8 IU/L) and 65.90 IU/L (SD 50.70) for Group IV (range from 10 to 146 IU/L). 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 ( $P > 0.05$ ). 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. This shows that S-ALP levels are significantly increased in OPMD irrespective of the tobacco habit

Table 1  
Salivary alkaline phosphatase in groups

Group	Mean	SD	<i>P</i>
I	19.00	12.37	<0.001*
II	8.50	3.35	
III	5.60	1.01	
IV	65.90	50.70	
Total	24.48	33.87	

## Discussion

Tobacco is a major etiological factor in the development of most of the OPMDs. Unfortunately, India is the second largest consumer of tobacco<sup>10</sup> 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.<sup>11</sup> The main carcinogens in tobacco products such as tobacco-specific nitrosamines, aldehydes, phenols, nitro compounds and polycyclic aromatic hydrocarbons<sup>12</sup> 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.<sup>13</sup> The potential for malignant transformation among OPMDs may vary from <1% to as high as 36%.<sup>14</sup> 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,<sup>15</sup> and its levels in saliva are known to increase in squamous cell carcinoma.<sup>16</sup> 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.<sup>16,17</sup> The normal levels of ALP in saliva range between 5.50 and 12.58 IU/L. The source of this enzyme in the oral cavity includes neutrophils, bacteria and oral epithelial cells.<sup>18</sup>

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 tobacco usage. This is similar to the values obtained by Maheswari.<sup>19</sup> 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.*<sup>20</sup> 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.<sup>21</sup> In addition, the physical, mechanical and chemical irritation caused by tobacco leads to keratosis,<sup>21</sup> 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-ALP values was observed by Maheswari.<sup>19</sup> 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<sup>22,23</sup> associated with the lesion. The rise in reactive oxygen species<sup>22</sup> induces cellular damage,<sup>23,24</sup> which leads to increased release of ALP in saliva. The increased rate of cellular turnover in OPMD,<sup>25</sup> 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<sup>26</sup> seen in association with OPMD could also be another contributing factor for the high levels of S-ALP observed.

## 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.

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