

**ORIGINAL RESEARCH****Evaluation of salivary alkaline phosphatase levels in tobacco users, non users and individuals with OPMD****<sup>1</sup>Dr Vandana Katoch, <sup>2</sup>Dr Narinder Singh**<sup>1</sup>Reader, Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Sehora, Jammu, Jammu and Kashmir, India<sup>2</sup>Consultant Paediatric, J&k Health Services, India**Correspondence:**

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**Email:**[vandanakatoch1@gmail.com](mailto:vandanakatoch1@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. The present study was conducted to evaluate the levels of salivary alkaline phosphatase in tobacco users, non users and individuals with OPMD.

**Material & methods:** The present study was conducted to evaluate the levels of salivary alkaline phosphatase in tobacco users, non users and individuals with OPMD. 80 participants were included in the study aged between 18 and 75 years who were categorized into four groups. A volume of 3 ml of unstimulated saliva was collected and samples were then centrifuged for the estimation of S-ALP levels. Data obtained were subjected to statistical analysis.

**Results:** The mean values for S-ALP were found to be about 19.23IU/L for Group I, 8.01IU/L for Group II, 4.56 IU/L for Group III and 65.36 IU/L for Group IV. Comparison of S-ALP between the groups showed a statistically significant difference ( $P < 0.001$ ).

**Conclusion:** The present study concluded that S-ALP was higher in patients with Oral potentially malignant disorders. So, S-ALP could be used as a reliable noninvasive biomarker in monitoring OPMD.

**Keywords:** S-ALP, Oral potentially malignant disorders, saliva, serum, tobacco

**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.<sup>1</sup> 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. In recent years, saliva which is an easily accessible oral fluid has gained acceptance as a diagnostic medium in many health conditions.<sup>2</sup> Saliva acts as an indicator and helps to identify immunological, inflammatory, endocrine, and metabolic biomarkers.<sup>3,4</sup> Several enzymes and antioxidants in saliva are known to offer protection against oxidative stress and cellular damage.<sup>5</sup> Alkaline

phosphatase (ALP) is a hydrolase intracellular enzyme that plays a key role in cellular metabolism. ALP is a glycoprotein produced within the periodontium and gingival crevice.<sup>4,6,7</sup> ALP is secreted by polymorphonuclear neutrophils, osteoblast, and periodontal ligament fibroblast at the time of inflammation, bone formation, and periodontal regeneration, respectively.<sup>8</sup> A 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.<sup>9</sup> The present study was conducted to evaluate the levels of salivary alkaline phosphatase in tobacco users, non users and individuals with OPMD.

### Material & methods

The present study was conducted to evaluate the levels of salivary alkaline phosphatase in tobacco users, non users and individuals with OPMD. Before the commencement of the study, ethical clearance was taken from the Ethical Committee of the institute. The study was explained to the participants and written informed consent was taken from the participants. 80 participants were included in the study 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 ( $n = 20$ )
- Group II – Individuals with the habit of chewing tobacco and without any lesion on intraoral examination ( $n = 20$ )
- Group III – Individuals with the habit of smoking and without any lesion on intraoral examination ( $n = 20$ )
- Group IV – Individuals with lesion on intraoral examination with the habit of smoking/chewing tobacco ( $n = 20$ ).

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. 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 were excluded from the study. A volume of 3 ml of unstimulated saliva was collected from all individuals by spitting method.<sup>10</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>11</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. Data obtained were subjected to statistical analysis. P value less than 0.001 was considered statistically significant.

### Results

80 participants were included in the study 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, Group II – Individuals with the habit of chewing tobacco and without any lesion on intraoral examination, Group III – Individuals with the habit of smoking and without any lesion on intraoral examination, Group IV – Individuals with lesion on intraoral examination with the habit of smoking/chewing tobacco).

#### Table 1: Comparison of salivary alkaline phosphatase between the groups

Groups	Mean± SD	p-value
Group I (n=20)	19.23±14.21	<0.001
Group II (n=20)	8.01±4.45	
Group III (n=20)	4.56±3.40	
Group IV(n=20)	65.36±53.27	

The mean values for S-ALP were found to be about 19.23IU/L for Group I, 8.01IU/L for Group II, 4.56 IU/L for Group III and 65.36 IU/L for Group IV. Comparison of S-ALP between the groups showed a statistically significant difference ( $P < 0.001$ ).

### Discussion

Saliva is an oral fluid that is produced by the major and the minor salivary glands. Saliva is composed mainly of 99% water while the remaining 1% comprises proteins, organic and inorganic constituents. It has been widely used in the detection of specific biomarkers which is of at most importance in disease diagnosis. Saliva contains various markers like enzymes, antibodies, immunoglobulins, hormones, bacteria and its products are all biomarkers which can be used in the diagnosis.<sup>11</sup>

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.<sup>7,10</sup>

The mean values for S-ALP were found to be about 19.23IU/L for Group I, 8.01IU/L for Group II, 4.56 IU/L for Group III and 65.36 IU/L for Group IV. Comparison of S-ALP between the groups showed a statistically significant difference ( $P < 0.001$ ).

Jain A et al conducted a study to measure S-ALP levels in smokers, nonsmokers, and people with OPMD and found 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.<sup>9</sup>

Menaka TR et al did a study to estimate and compare the levels of S-ALP among tobacco users, nonusers and in individuals with OPMD. 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. The study concluded that S-ALP could be used as a reliable noninvasive biomarker in monitoring OPMD.<sup>12</sup>

The increased S-ALP levels observed in OPMD cases could be secondary to the increase in the oxidative stress associated with the lesion. The rise in reactive oxygen species induces cellular damage, which leads to increased release of ALP in saliva. The increased rate of cellular turnover in OPMD 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 seen in association with OPMD could also be another contributing factor for the high levels of S-ALP observed.<sup>12</sup>

### Conclusion

The present study concluded that S-ALP was higher in patients with Oral potentially malignant disorders. So, S-ALP could be used as a reliable noninvasive biomarker in monitoring OPMD.

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