Original Article

Detection of Salivary Alkaline Phosphatase Levels in Smokers, Diabetic Patients, Potentially Malignant Diseases and Oral Malignant Tumours

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Aim: Alkaline phosphatase (ALP) is present in human and plays a key role in intracellular destructive processes and cellular damage. It has bicarbonate and phosphate ions, which help in buffering against acids. ALP activity is affected by smoking, which changes pH in oral environment and has harmful effects. Thus, the evaluation of ALP activity of smokers and healthy nonsmoker along with patients who were diabetic, potentially malignant, and malignant was carried out in this study. Materials and Methods: The study took place between 2016 and 2017. A total of 150 smokers, non-smokers, and patients who were diabetic, potentially malignant, and malignant were included. Collection of unstimulated whole saliva was done from each participant, and salivary ALP levels were measured by spectrophotometric assay. Results: Mean salivary ALP levels were significantly higher in smokers compared to those in nonsmokers. Mean ALP levels were also increased in patients who were diabetic, potentially malignant, and malignant compared to those in controls. Conclusion: ALP levels were significantly higher among smokers when compared to a healthy control group. Oral tissue function and side effects among smokers can be evaluated by these salivary enzyme alterations, which can serve as biomarkers for the diagnosis of any disease process. These salivary alterations could potentially be used as biochemical markers for the evaluation and early diagnosis. The higher levels were also found in patients who were diabetic, potentially malignant, and malignant, and ALP levels may also be used as biomarkers for the evaluation of the disease process.

KEYWORDS: Alkaline phosphatase, diabetic, saliva, smokers

INTRODUCTION

Early detection of a disease determines the prognosis to a large extent. Saliva is a suitable alternative to blood as it is easy to collect and noninvasive and can serve as a tool for early detection of any disease.[1] This

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research is focused on early detection by using saliva as a tool for early diagnosis. Its advantages are as follows: readily available, safe, and noninvasive.\textsuperscript{13} Saliva plays a vital role in defining the biomarkers for cancer risk as it moistens the complete oral cavity and also reflects many oral diseases including oral cancer.\textsuperscript{3} Various medical conditions such as malignancies, metabolic diseases, infections, and autoimmune disorders can all be detected early by salivary biomarkers, and with respect to dental aspect, salivary alkaline phosphatase (ALP) is of prime importance.\textsuperscript{1}

ALP is a phosphor-hydrolytic enzyme balancing the remineralization–deminerlization cycle as it is primarily involved in calcium and phosphate binding. It seems that the function of this protein relatively depends on the salivary pH and buffering capacity.\textsuperscript{4}

Development of various life-threatening diseases occurs due to cigarette smoke owing to the presence of components such as pyridine alkaloids, aromatic hydrocarbons, and combustion gases. The deleterious effects of smoking will affect the oral mucosa, associated with salivary changes.\textsuperscript{5} Periodontal disease is a common feature of diabetes mellitus (DM). It is a metabolic disorder affecting various systems due to changes in the metabolism of protein, carbohydrate, and lipids. Cardinal biochemical feature of this disease is elevated blood glucose (hyperglycemia). Oral and systemic health is also affected when there are imbalances in the quantity or quality of saliva.\textsuperscript{6}

Potentially malignant diseases (PMD) of the oral mucosa are relatively common, which include oral leukoplakia, erythroplakia, and oral submucous fibrosis. Leukoplakia is one of the most common potentially malignant lesions of the oral mucosa. Diagnosis is made based on clinical history and examination of the lesion, though biopsy is necessary for the confirmation of the diagnosis. Biopsy is the gold standard for cancer diagnosis currently, but the process of biopsy has few pitfalls when diagnosing early-stage lesions. There are various noninvasive techniques in detecting oral premalignancies, which include vital tissue staining with toluidine chloride, various visualization adjuncts, which include ViziLite, Microlux DL system, Orascoptic DK system, and VELscope system and cytopathology by OralCDx Brush Test system. But these techniques are expensive, and the patients will not be willing to afford it for an asymptomatic lesion. Several studies have been conducted to analyze the salivary biomarkers in oral cancer and oral precancer.\textsuperscript{7}

A limited amount of studies have been undertaken on salivary ALP activity in smokers and patients who are diabetic. The aim of the study was to evaluate and compare the salivary ALP in patients who are nondiabetic and diabetic. The detection of potentially malignant disorders and oral cancers in any stage can be done by information of physiological and pathological conditions provided by these biomarkers.\textsuperscript{7} From all these in addition to the aforementioned diabetic and smokers, the salivary ALP levels were also considered in individuals who were potentially malignant and malignant.

**Materials and Methods**

The patients who presented to the department of oral surgery and oral pathology were included in the study. A total of 150 individuals between the age-group of 30 and 60 years were considered, and were subdivided into six study groups with Group A including 25 nonsmokers and nondiabetic individuals as controls, Group B including 25 individuals with diabetes who were nonsmokers, Group C including 25 individuals who were nondiabetic smokers, Group D including 25 smokers who were diabetic, Group E including 25 individuals with leukoplakia with 12 among them were smokers, and Group F including 25 patients with oral squamous cell carcinoma (OSCC). Inclusion criteria in the study consist of healthy individuals without any habits and systemic disease. Exclusion criteria include individuals without any periodontitis and systemic diseases such as liver and renal diseases.

Having obtained written consent from the patient, collection of 5 mL of unstimulated saliva was done from the patients under aseptic conditions, and with prior instructions to not to consume food for 2 h before collection. The saliva was collected through the split technique, and the patient was explained about the technique. The saliva samples were then subjected to centrifugation at 3000 rotations per minute for 15 min to separate the supernatant saliva. The 20 μL of the remaining sample was mixed with the ALP reagent in the ERBA Mannheim kit (London, UK) [Figure 1], and the levels were analyzed through the auto-analyzer [Figure 2]. The readings that were obtained on the screen of the analyzer were then noted.

**Results**

The statistical test used to compare the data was one-way analysis of variance (ANOVA) and post hoc Tukey test, and the results showed a significant correlation. Significant difference in the ALP values in the six groups was evident; highest was reported in the OSCC group > PMD > smoker with diabetes > nonsmoker with diabetes > smoker and nondiabetic > nonsmoker.
and nondiabetic. Nonsmoker and nondiabetics are significantly different from all the groups; however, nonsmoker and diabetics are significantly different only from the OSCC group as seen in the post hoc Tukey test.

The salivary ALP levels were significantly increased in leukoplakia individuals with smoking and OSCC compared to those in controls. Also, significantly increased activity was in smokers with the diabetic group when compared to that in the controls, diabetics, and smokers. Multinomial logistic regression analysis shows that male gender has 2.979 odds of having a malignant condition compared to females. Smoking and diabetes are not significantly influencing the onset of PMD or the malignant condition compared to the no lesion group. Salivary ALP and age are significantly associated with the occurrence of the PMD, and ALP is significantly associated with the malignant [Tables 1–4].

**DISCUSSION**

Diagnosis of disease by using saliva as a tool is an effective modality for the early intervention, prognostic outcome, and post-therapy monitoring of the disease status. The presence of entire library of enzymes, hormones, proteins, antibodies, cytokines, and antimicrobial constituents makes saliva a complex fluid with great diagnostic value. The route of entry of these components into saliva through blood is either by transcellular, passive intracellular diffusion, and active transport or by paracellular routes such as extracellular ultrafiltration within the salivary glands or from the gingival crevice. A noninvasive collection technique of the of saliva in smaller sample aliquots associated with good patient cooperation makes saliva better alternative over serum or any tissues of the body. Other than these advantages, it is also cost-effective, easy to store and transport, shows great sensitivity, and shows correlations with levels in blood. Salivary biomarkers are now much in consideration as new technologies are evolving for their usage in various disease conditions.[8]

Liver and bones contribute to majority of ALP in the body and minor amounts from kidneys, intestines,
The alkaline environment created by the group of enzymes of ALP helps to catalyze the hydrolysis of phosphate esters. It is thought that lipid transport in intestine and calcification mechanisms in bone are aided by ALP, though complete metabolic role is not yet ascertained. Nucleotides, which are building blocks for DNA and proteins, are altered by ALP, wherein phosphates groups are cleaved from its structure. [9]

Alteration in activity enzymes such as ALP, serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase, is a major feature in DM, though complete metabolic role is not yet ascertained. Nucleotides, which are building blocks for DNA and proteins, are altered by ALP, wherein phosphates groups are cleaved from its structure. [9]

A significant increase in the salivary ALP concentrations between smoker and nonsmokers was observed in our study, which is similar to the findings of a study by Kareem and Ibrahim [11].

### Table 2: Post hoc Tukey subgroup analysis

| Comparison of the two groups | Mean difference (I – J) | Std. error | P value (significant if <0.05) | 95% Confidence interval | Lower bound | Upper bound |
|-----------------------------|-------------------------|------------|--------------------------------|-------------------------|-------------|-------------|
| Nonsmokers nondiabetics     | -17.787600              | 2.278912   | <0.001                         | -24.37017               | -11.20503   |
| Smokers nondiabetics        | -11.487600              | 2.278912   | <0.001                         | -18.07017               | -4.90503    |
| Smoker diabetics            | -18.879600              | 2.278912   | <0.001                         | -25.46217               | -12.29703   |
| PMD                         | -19.689200              | 2.278912   | <0.001                         | -26.27177               | -13.10663   |
| OSCC                        | -31.607600              | 2.278912   | <0.001                         | -38.19017               | -25.02503   |
| Nonsmokers diabetics        | 6.300000                | 0.069      |                                | -0.28257                | 12.88257    |
| Smoker diabetics            | -1.092000               | 2.278912   | 0.997                          | -7.67457                | 5.49057     |
| PMD                         | -1.901600               | 2.278912   | 0.961                          | -8.48417                | 4.68097     |
| OSCC                        | -13.820000              | 2.278912   | <0.001                         | -20.40257               | -7.23743    |
| Smokers nondiabetics        | -7.392000               | 2.278912   | 0.018                          | -13.97457               | -0.80943    |
| PMD                         | -8.201600               | 2.278912   | 0.006                          | -14.78417               | -1.61903    |
| OSCC                        | -20.120000              | 2.278912   | <0.001                         | -26.70257               | -13.53743   |
| Smoker diabetics            | -0.809600               | 2.278912   | 0.999                          | -7.39217                | 5.77297     |
| PMD                         | -12.728000              | 2.278912   | <0.001                         | -19.31057               | -6.14543    |
| OSCC                        | -11.918400              | 2.278912   | <0.001                         | -18.50097               | -5.33583    |

### Table 3: Multinomial logistic regression analysis

| Case processing summary | N | Marginal percentage |
|-------------------------|---|---------------------|
| Group                   |   |                     |
| No lesion               | 100 | 66.7%               |
| Potentially malignant lesion | 25 | 16.7%               |
| Malignant condition     | 25 | 16.7%               |
| Gender                  |   |                     |
| Female                  | 55  | 36.7%               |
| Male                    | 95  | 63.3%               |
| Diabetes                |   |                     |
| Present                 | 70  | 46.7%               |
| Absent                  | 80  | 53.3%               |
| Smokers                 |   |                     |
| Present                 | 79  | 52.7%               |
| Absent                  | 71  | 47.3%               |
| Valid                   | 150 | 100.0%              |
| Missing                 | 0   |                     |
| Total                   | 150 |                     |
| Subpopulation           | 145 |                     |

[a] The dependent variable has only one value observed in 144 (99.3%) subpopulations

were increased in diabetic when compared to those in controls. The results were also consistent with the study conducted by Sridharan et al.[10]

The effect of smoking on ALP may be due to an altered imbalance between the free oxygen radicals and imbalance in the antioxidant levels. Till date, many
Biomarkers have been analyzed in both serum and saliva and are equally sensitive as serum. In this study group, the patients with leukoplakia with smoking habits showed higher ALP levels compared to patients who were nonsmoker and with leukoplakia. The duration of the smoking habit in these patients was 10–15 years.[12]

Biochemical levels found in the saliva of cancer patients should also include ALP evaluation as it helps in the early diagnosis and also it is inexpensive. Elevated levels of ALP are frequently observed in advanced cancers.[13] Gupta [2] and Shetty et al.[1] found raised serum ALP (<0.05) in head and neck cancers. The mean value of ALP was significantly higher than control in OSCC and leukoplakia. In this study, instead of serum, salivary levels were considered, which showed raised ALP in patients with both leukoplakia and OSCC, which is similar to the findings of a study by Prakash et al.[14] The advanced stage of disease in patients of our study led to higher incidence of raised ALP levels. These levels may also be used as a prognostic biomarker for both potentially malignant and malignancy.

**Conclusion**

Saliva is a noninvasive technique and is as sensitive as serum. The salivary ALP levels may predict the effects of smoking and diabetes. The ALP levels are increased in potentially malignant and malignant lesions and thus can be used as biomarker for the early diagnosis and disease process.

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**Conflicts of interest**

There are no conflicts of interest.

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