INTRODUCTION

Saliva is an oral fluid that has been used as a diagnostic tool in medicine and dentistry.[1] 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. Several enzymes evaluated for the early diagnosis of periodontal disease are lactate dehydrogenase, alkaline phosphatase (ALP), acid phosphatase, aspartate aminotransferase and alanine aminotransferase.[2] Sampling technique for GCF collection is a time-consuming process and is a difficult procedure. This was first recognized by Ishikawa and Cimasoni, who demonstrated a level of ALP enzyme in GCF and showed a significant correlation between ALP concentration in GCF and pocket depth.[3] So easy, non-invasive, less time-consuming, chairside technique like salivary-ALP (S-ALP) analysis from saliva samples was preferred in our study.

The by-products of cigarette smoking such as carbon monoxide, hydrogen cyanide and benzopyrene are toxic.[4] Cigarette smoking is known to be one of the major causes

ABSTRACT

Background: Alkaline phosphatase (ALP) is a hydrolase intracellular enzyme participating in the metabolic processes of cells. Rise in salivary ALP (S-ALP) levels reflects inflammation and destruction of healthy tissues suggesting it as a clinical biomarker. S-ALP is used in analyzing the severity of the disease occurrence in smokers and nonsmokers who are diabetic and nondiabetic. S-ALP levels are analyzed using autoanalyzer in 40 patients who visited our department. Aims and Objectives: To determine the levels of S-ALP in diagnosing potentially malignant conditions and debilitating diseases in early stages of inflammation and altered cellular metabolism. Materials and Methods: The study groups include: (1) Group A - 10 smokers who are diabetic. (2) Group B - 10 smokers who are nondiabetic. (3) Group C - 10 nonsmokers who are diabetic. (4) Group D - 10 nonsmokers and nondiabetic as control. Unstimulated saliva samples are collected and run in auto-analyzer with ALP enzyme reagent to analyze ALP levels. Comparison is made between all the four groups. Results: Results were statistically significant with increased activity of ALP levels in saliva from Group A when compared to Group D. The results are Group A > Group B > Group C > Group D. The results also revealed significant raise in levels of ALP levels in saliva from smokers when compared to diabetes. Thus explaining adverse effects of smoking. Conclusion: S-ALP can be considered to be the biomarker for evaluating adverse effects of smoking, diabetes and other debilitating diseases in early stages.

Key words: Diabetic, salivary alkaline phosphatase levels, smokers

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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.\textsuperscript{[5,6]} 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 ($O_2^-$) system and phosphatases.\textsuperscript{[7]} Diabetes is one of the systemic disorders that has adverse effects on various human organic systems leading to nephropathy, delayed wound healing, etc. The S-ALP levels were higher among diabetics than nondiabetics.\textsuperscript{[3]} Intracellular enzymes are increasingly released from the damaged cells of periodontal tissues due to diabetes into the GCF and saliva.

S-ALP is one of the sensitive markers for the early detection of oral carcinoma. Salivary diagnosis can be performed in all the dental institutions to assess the malignant risk potential of potentially malignant disorders and thus quality of life of patients can be improved.\textsuperscript{[8-10]}

Based on these reviews, this study is conducted to evaluate the role of S-ALP in three groups which includes diabetics, smokers, and diabetics who smoke.

**MATERIALS AND METHODS**

The study included a total of 40 patients between the age group of 30 and 50 years who visited our department. Group A included 10 smokers who were diabetic, six among them were diagnosed with leukoplakia. Group B included 10 smokers who were nondiabetic, two among them were associated with leukoplakia. Group C included 10 nonsmokers who were diabetic. Group D included 10 nonsmokers and nondiabetic as control. Patients with systemic diseases such as Pagets, renal failures and liver cirrhosis where there will be raise in ALP were all excluded from the study. Patients who were diagnosed with periodontitis clinically based on probing depth, bleeding gums and radiographical bone loss were excluded from the study.

Ethical clearance was obtained from the institutional review board and consent forms from patients were also taken.

A volume of 5 ml of unstimulated saliva samples were collected in sample container from all the participants. Subjects were instructed not to take food for 2 h prior to saliva collection. After rinsing the mouth, patients were asked to collect saliva in the floor of the mouth and then spit into a sample container. The sample was then centrifuged at 3000 rpm for 15 min to remove debris. The resultant supernatant saliva was separated, 20 µl of the sample was mixed with 1000 µl of ERBA Mannheim kit ALP reagent for the estimation of S-ALP levels in auto-analyzer. This was based on kinetic method recommended by International Federation of Clinical Chemistry (Figures 1 and 2). The sample mixed with reagent were run in auto analyzer [Figure 3]. The readings obtained on the screen of analyzer were noted [Figure 4].

**RESULTS**

One-way ANOVA was performed to compare data among all groups and found that results were statistically significant with increased activity of ALP levels in saliva from smokers with diabetes group when compared to control, diabetics and smokers. According to some studies, in diabetic patients, there will be raise in S-ALP levels due to bone loss. In our study, smokers had significant raise in S-ALP levels when compared to diabetic and control groups.

Three cases were diagnosed as homogenous leukoplakia, of three cases two patients were using bidi and one patient was using cigarette with oral medication for diabetes. Duration of smoking ranging from 15 to 40 years exhibiting higher levels of S-ALP (74.48 µ/l) when compared to smokers without oral lesions (27.81 µ/l) [Tables 1-3], Figure 5.

Among 20 diabetic patients in both Groups C and D, 19 were on oral medication and only one person was not using any medication showing high S-ALP level irrespective of duration.

**DISCUSSION**

Interest in using saliva as a diagnostic medium has been increasing in the last 10 years.\textsuperscript{[3]} Salivary components for diagnosis include enzymes, immunoglobulins, hormones of host origin, bacteria and bacterial products, ions and volatile compounds. Enzymes in saliva have been studied as markers of periodontal disease and these are derived from cells in the salivary glands, macrophages from gingival sulcus or pockets, polymorphonuclear (PMNs) leukocytes, epithelial cells from oral cavity and microorganisms.\textsuperscript{[4]}

![Figure 1: Alkaline phosphatase reagent](image-url)
Association of Salivary alkaline phosphatase with smoking, diabetes and PMDs

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ALP is a membrane-bound glycoprotein found on most cell membranes in the body and physiologically occurs during bone formation in developmental stages. It is produced by many cells within the periodontal environment, the principal source being PMNs leukocytes, bacterial fibroblast and osteoblast activity which is disturbed due to diabetes, smoking, etc., pathologically. ALP is one of the potentially powerful markers of periodontal disease activity and ALP levels increases in periodontal diseases. Thus, we excluded periodontal disease patients who were diagnosed clinically and radiographically with pockets and bone loss. This is to avoid false positive results and to evaluate ALP diagnosing sensitivity in diabetes and smoking alone.

Toxic products due to smoking levels depend on the balance between their rates of production and their rates of clearance by the endogenous antioxidant systems, including superoxide dismutase (SOD), catalase (CAT), the glutathione (GSH) redox cycling enzymes, GSH-Px, reductase and GSH itself. One of the principal reactive oxygen species produced in aerobic organisms is \( \text{O}_2^- \), which is highly cytotoxic. With the cytotoxicity of this oxidant, exposure to cigarette smoke results in increased levels of antioxidant enzymes, such as CAT, copper/zinc SOD, Px and GSH-Px. The higher reactive \( \text{O}_2^- \) is converted to \( \text{H}_2\text{O}_2 \) by SOD. CAT, Px, or GSH-Px can, in turn, convert \( \text{H}_2\text{O}_2 \) to molecular oxygen and water. Under physiologic conditions, these systems tend to maintain a stable state called redox homeostasis. The imbalance between the formation of free oxygen radicals and inactivation of these species by antioxidant is capable of causing damage to various cellular and extracellular constituents. At the same time, S-ALP evaluation which is altered due to antioxidant imbalances can act as alarm in smokers and diabetics. Cigarette causes destruction of vascular collagen.

This study showed higher S-ALP levels in diabetics, smokers than controls, explaining the cellular metabolic alterations. Furthermore, S-ALP levels in smokers with diabetics group significantly proposes there exists some role between salivary enzymes and harmful habits like smoking to which diabetes shows additive destructive behavior. Noticeable raise of S-ALP in premalignant disorders observed in sample explains that premalignancy related adverse effects can also be analyzed by simple sialodiagnosis. Further studies on large populations...
are needed to confirm the reliability of these parameters and to determine their effect systemically due to smoking, premalignancy and malignancy.

**CONCLUSION**

On the basis of the results of this study, it can be concluded that the S-ALP enzyme was significantly greater in diabetes mellitus, smokers and subjects with potentially malignant disorders without any periodontitis compared to systemically healthy individuals. We emphasize that screening of premalignancies and malignant lesions can be made by measuring the S-ALP levels. The technique is also feasible, simple and has a convenient approach.

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**Conflicts of interest**

There are no conflicts of interest.

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