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Estimation Of Salivary Alkaline Phosphatase Levels In Smokers And Tobacco Chewers Associated With Diabetes

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ABSTRACT: Background:- Alkaline phosphatase is the group of hydrolytic enzymes that are involved in the cellular metabolism. Alkaline phosphatase in saliva is used as an indicator of gingival inflammation, bone metabolism and other oral lesions like premalignancies and oral cancer.
Aim and objective: To estimate the levels of Salivary Alkaline Phosphatase (S-ALP) in smokers and tobacco chewers associated with diabetes and diabetics without any habits. Materials and Methods: The study includes 30 cases and 10 control. 30 cases divided in group A, B and C and control group D: (1) Group A - 10 smokers who are diabetic. (2) Group B - 10 tobacco chewers who are diabetic. (3) Group C - 10 nonsmokers and non-tobacco chewers who are diabetic. (4) Group D - 10 nonsmokers, non-tobacco chewers and nondiabetic as control. Saliva was collected from the subjects of the above mentioned groups and the levels of S-ALP were estimated using a semi auto- analyzer and comparison was made. Results: The result reveals increase in the alkaline phosphatase levels in saliva of smokers and tobacco chewers with diabetes group (Group A, B). Non-smokers and non-tobacco chewers with diabetes group (Group C) had higher level compared to the control group (Group D). Conclusion: Salivary alkaline phosphatase is a clinical biomarker that is used to determine the oral diseases due to adverse habits and diabetes at its early stage.

Keywords: Salivary alkaline phosphatase, Smokers, Tobacco Chewers, Diabetics.

1. INTRODUCTION

Saliva is an important and most ideal diagnostic tool that has been used from time ahead. It is inexpensive, noninvasive, and easy to use. Sample collection is possible with minimal patient discomfort which is what makes it more acceptable to the patient as well as the clinician in both medicine and dentistry [1]. For the past two decades, salivary diagnostics have been in use to detect oral and also systemic diseases. It contains most of the serum components that enter the saliva through passive intracellular diffusion. The serum components found in saliva are

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enzymes, hormones, microbial cells, and their products, antibodies, etc[2]. Saliva is an important yet complex body fluid which is very essential for oral health and it is the first biological fluid that is exposed to the tobacco products which contains several toxins that account for the functional and structural changes in saliva [3]. For this reason, saliva samples were used for this study.

The carcinogens in cigarette smoke are from multiple chemical classes, including polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, aromatic amines, aldehydes, volatile organic hydrocarbons, and metals. Apart from these well-established carcinogens, others have been less thoroughly investigated. These include alkylated PAHs, oxidants, free radicals, and ethylating agents [4] and carcinogens like nonvolatile alkaloid-derived Tobacco Specific Nitrosamine A (TSNAs), NNN, NNK, volatile N-nitrosamines produced from tobacco chewing [5]. These toxic components can be a predisposing factor to different systemic disorders, such as cardiac diseases, cancers, precancerous lesions and pulmonary disorders [6]. Diabetes mellitus is a metabolic disorder characterized by high blood glucose level associated with other manifestations. It has adverse effects on various human organic systems leading to neuropathy, nephropathy, retinopathy, delayed wound healing, periodontitis, stroke and myocardial infarction [7].

According to the National Institutes of Health (NIH), "a biomarker is an objectively measured and evaluated indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention" [8]. Salivary biomarkers has been extensively used to aid in the diagnosis of oral and systemic diseases such as malignancies, autoimmune disorders, infections and metabolic diseases. Among the salivary biomarkers, alkaline phosphatases (S-ALP) has been of prime interest, especially from the dental aspects [9]. S-ALP is a clinical biomarker and its increased level suggests any oral lesion in its initial stage where there is altered cellular metabolism [6]. This study is conducted to evaluate the role of S-ALP in three groups which includes smokers and tobacco chewers with diabetes and diabetics without any habits.

2. MATERIALS AND METHODS

The study included a total of 40 subjects with 30 case and 10 control between the age group of 20 and 60 years. Group A included 10 smokers who were diabetic. Group B included 10 tobacco chewers who were diabetic. Group C included 10 nonsmokers and non-tobacco chewers who were diabetic. Group D included 10 nonsmokers, non-tobacco chewers and nondiabetic as control. Patients with systemic diseases such as Paget's disease and other bone pathology, renal failures and liver cirrhosis where there will be raise in alkaline phosphatase were all excluded from the study.

Ethical clearance was obtained from the institutional review board and the consent was obtained.

A volume of 2 ml of unstimulated saliva samples was collected in sample container from all the participants since unstimulated saliva contains high concentration of markers compared to stimulated saliva. Subjects were instructed not to take food 2 hours prior to saliva collection. The subjects were asked to rinse their mouth with plain water and 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 5 min to remove debris [Fig- 1]. The resultant supernatant saliva was separated [Fig- 2], 0.02 ml of the sample was mixed with 1ml of Beacon- Liquizyme kit ALP reagent (Navsari, India) [Fig- 3] for the estimation of S-ALP levels in semi auto-analyzer. This was on

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the basis of kinetic method recommended by International Federation of Clinical Chemistry. The samples mixed with reagent were run in semi auto analyzer [Fig- 4 and 5]. The readings obtained from the analyzer were noted [Fig- 6].

3. RESULT

One-way ANOVA was performed to compare data among all groups and found that results were statistically significant. According to this study, Group A (Smokers with diabetes) has the highest salivary level of alkaline phosphatase when compared to other groups- Group B (Tobacco chewers with diabetes), Group C (Diabetics with no habits) and Group D (Control).
Group B (Tobacco chewers with diabetes) has high salivary level of alkaline phosphatase when compared to Group C (Diabetics with no habits) and Group D (Control), but less compared to Group A (Smokers with diabetes). According to this study, the level of salivary alkaline phosphatase in Group A is high compared to Group B suggesting increased tissue damage in Group A.
Group C (Diabetics with no habits) has high salivary level of alkaline phosphatase compared to Group D (Control), but less compared to Group A (Smokers with diabetes) and Group B (Tobacco chewers with diabetes). This explains that the level of salivary alkaline phosphatase is influenced by the presence of habits.
Group D (Control) has lowest level of salivary alkaline phosphatase compared to the other groups- Group A (Smokers with diabetes), Group B (Tobacco chewers with diabetes) and Group C (Diabetics with no habits). This explains that the salivary alkaline phosphatase level is influenced by the presence of habits and diabetes [Table-1, 2] [Fig- 8, 9]

4. 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 [10]. Salivary diagnostics is an efficient means of diagnosis of a disease throughout its course. Salivary components may vary in the concentration depending upon an individual's health or disease status and monitoring these data forms the foundation of salivary diagnosis. Therefore, salivary diagnostics has been emerging as a gold standard in the field of diagnosis for its efficacy and advantages [11].
By the definition, a biomarker invariably helps in diagnosing a disease or any absence of normal biological activity at an early stage and hence improving the patient's quality of life [8].
The tobacco products contain many toxic chemicals, carcinogens and free radicals in stable and unstable form. These free radicals are in huge amount causes local irritation and damage to tissues. The tissue damage caused leads to the production of biomarkers [12]. The biomarkers produced by the tissues in saliva is an important constituent for the detection of tissue inflammation, premalignacy and oral squamous cell carcinoma [13, 14] .
Free radical formation in diabetes by non-enzymatic glycation of proteins, increase in lipid peroxidation and oxidation of glucose leads to enzymatic damage, cellular machinery, and also

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increase in insulin resistance due to oxidative stress that in turn leads to the production of biomarkers [15].

One of the important and sensitive biomarkers for the early detection of oral squamous cell carcinoma is Salivary Alkaline phosphatase (S-ALP) which has been used in this study [16].

The limitation of this study is the inclusion of subjects with periodontitis since this is an institutional study. The patients visiting our institution are of low socio- economic status and were of relatively poor oral hygiene.

In this study, smokers and tobacco chewers with diabetes had significant raise in salivary alkaline phosphatase levels when compared to diabetic without habit group and control groups [Table 1 and 2] [Fig- 8 and 9]

5. CONCLUSION

This study showed higher salivary alkaline phosphatase levels in diabetics with habits and diabetics without habits than controls, explaining the cellular metabolic alterations. It can be considered as an important biomarker for evaluating the adverse effects of smoking, tobacco chewing, diabetes and other debilitating diseases in early stages. It is emphasized that screening of saliva in individuals with habits with or without diabetes can be made by measuring the salivary alkaline phosphatase levels to know the extent of tissue damage, and the probability of the individual to develop oral malignancy. The technique is also feasible, simple and has a convenient approach.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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TABLES

Table-1 Mean S-ALP levels in each group and there comparisons

Group	**n**	**Mean ALP**	**SD**	**SE**	**95% CI for mean**		**Maximum**	**Minimum**
					Lower Bound	**Upper Bound**		
Smokers with Diabetes	10	21.5	17.454	5.519	9.07	34.03	54	8.7
Tobacco chewers with Diabetes	10	19.31	13.928	4.404	9.35	29.27	45	7.8
Diabetics	10	10.6	6.886	2.602	6.52	14.68	26	5.4
Control	10	5.72	0.998	0.315	5.01	6.43	7.7	4.2
Total	40	14.28	7.399	3.699	11.91	16.65	21.5	5.72

n- Number, ALP- Alkaline phosphatase, SD- Standard Deviation. SE- Standard Error, CI- Confidence Interval

Table-2 One way ANOVA

Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	2754.943	7	393.5633	4.201257	0.00219788	2.31274118
Within Groups	2997.68	32	93.67751			
Total	5752.623	39				

ANOVA- Analysis Of Variance, SS- Sum of Square, df- degree of freedom, MS- Mean Square

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FIGURES

Figure-1 Samples collected are centrifuged at 3000 rpm for 5 minutes

Figure-2 Centrifuged samples in Eppendorf tubes

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Figure-3 Alkaline phosphatase reagent

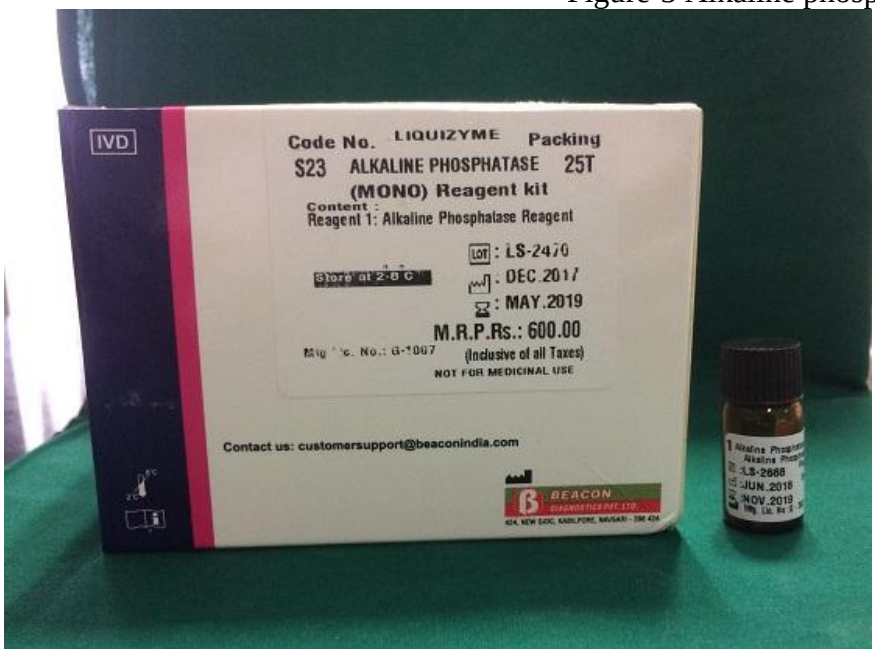

Figure-4 Samples transferred to the alkaline phosphatase reagent

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Figure-5 Testing the samples in analyzer

Figure-6 Salivary alkaline phosphatase values assessed in semi auto analyzer

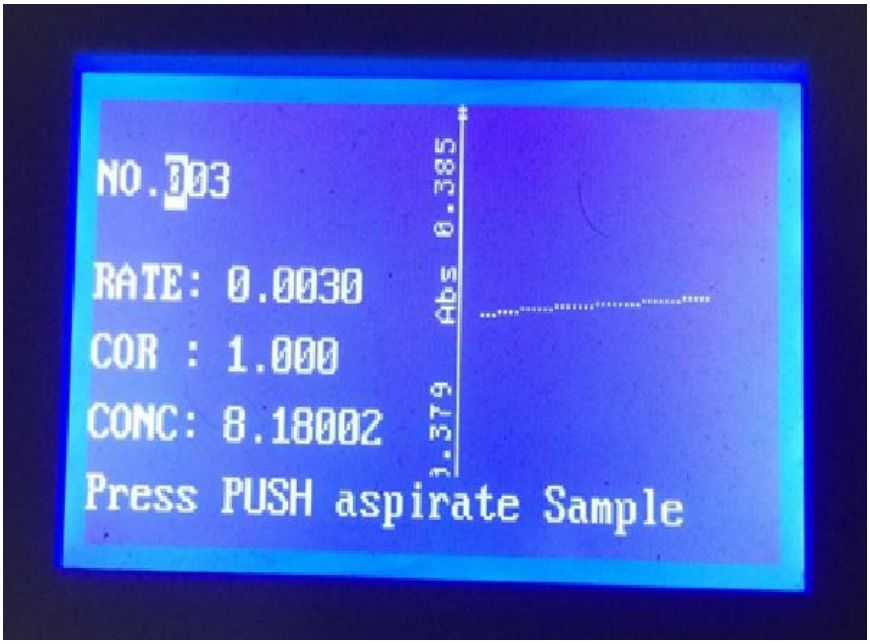

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Figure-7 Mean Level of Salivary Alkaline Phosphatase corresponding to each group

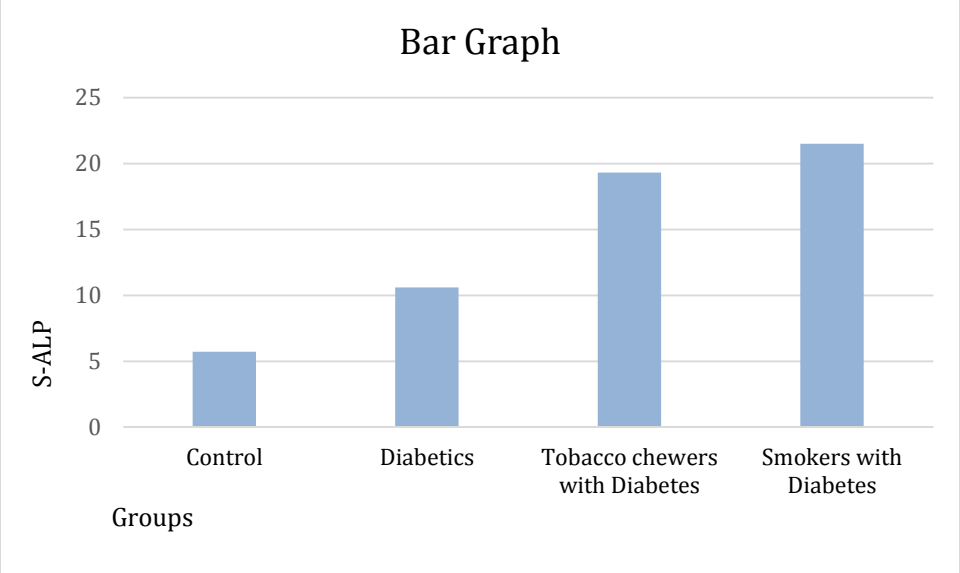

Figure 8 Box Plot