Salivary Alkaline Phosphatase as a Noninvasive Marker for Periodontal Disease in Children with Uncontrolled Type 1 Diabetes Mellitus

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Objective: *The aim of this pilot study was to determine whether salivary alkaline phosphatase levels can be a non invasive marker for early inflammatory periodontal disease in children with uncontrolled type 1 diabetes mellitus.* ***Study design:*** *10 healthy children (group 1), 10 children with recently diagnosed type 1 diabetes mellitus (group 2) and 10 children with type 1 diabetes mellitus for more than 4 years (group 3) were recruited for the study. All three groups were matched for age, gender and socioeconomic status. Periodontal health was assessed by plaque index, gingival index and probing pocket depth. Metabolic status was assessed by glycosylated hemoglobin levels, salivary alkaline phosphatase levels were determined by spectrophotometer. Data was analyzed by Kruskal Wallis ANOVA, Mann-Whitney U test and Spearman's rank correlation method.* ***Results:*** *Salivary alkaline phosphatase levels correlated significantly with the periodontal parameters in the diabetic group. An increase in salivary alkaline phosphatase levels increased with increased values of gingival index and probing pocket depth. Group 3 showed greater correlation than group 2 and group 1. At p value p<0.05.* ***Conclusion****: The glycemic status of the children affects the periodontal disease parameters. Salivary alkaline phosphatase levels could be a useful tool in analyzing periodontal status of children with uncontrolled type I diabetes mellitus.*

Key words: type 1 diabetes mellitus, alkaline phosphatase, periodontal disease, saliva.

INTRODUCTION

Type 1 Diabetes mellitus (type 1 DM) results from the autoimmune destruction of pancreatic islet cells, eventually leading to the loss of insulin production, and is usually diagnosed in children and young adults, [1] in contrast, the onset of type 2 Diabetes mellitus (T2DM) is in adulthood, and it is characterized by an increase in insulin resistance associated with a varying inability of pancreatic b-cells to secrete sufficient amounts of insulin to compensate. Besides representing a risk factor for numerous organ complications [2], DM is an important and independent risk factor for development of gingivitis and periodontitis. In fact, age is an element that influences many factors forming the basis of the interrelationship between DM and periodontal disease [3] length of DM conditions the severity of periodontal disease[4].

Diabetes and periodontal disease are two chronic diseases that have long been considered to be biologically linked. A large amount of case reports, cross sectional studies, longitudinal studies, and reviews report the adverse effects of diabetes on the onset, progression, and severity of periodontal disease [5, 6]. The prevalence of periodontitis in diabetic subjects is estimated to be double or even triple the number in the normal population [1] It has been suggested that hyperglycemia and resultant advanced glycation end product formation, which is one of the several pathways that is thought to lead

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to the classic microvascular and macrovascular complications of diabetes, are also involved in the patho physiology of periodontitis in diabetic subjects. Aspriello[7] in his study showed an association between Type 1 DM and an increased risk for periodontal destruction in children less than 10 years of age and further highlights the necessity of treating periodontitis during the early stages of T1DM development. He also emphasized the fact that the gingival crevicular fluid levels of IL-1β and TNF-α were higher in the type 1 DM patients of longer duration than those patients who were recently diagnosed.

It is postulated that alkaline phosphatase (ALP) could serve as a prognostic predictor, as an adjunct to the routine methods used for determination of the disease activity and has a direct influence on the diagnosis, therapy, and prognosis of periodontitis[8]. ALP is calcium and phosphate binding glycoprotein and a phosphor-hydrolytic enzyme. It is produced by many cells such as polymorphonuclear leukocytes (PMNLs), osteoblasts, macrophages, and fibroblasts within the area of the periodontium and gingival crevice. [9]. ALP is considered to be an important indicator of osteoblastic activity. ALP is detected in the parotid, submandibular, and minor salivary glands, as well as in desquamated epithelial cells, leukocytes, and bacteria from dental plaque. The presence of ALP in the saliva is usually indicative of inflammation and destruction of the periodontal tissues. The level of ALP is positively correlated with the severity of the periodontal disease[10]. As far as the periodontium is concerned ALP is very important enzyme as it is a part of normal turnover of periodontal ligament, root cementum and alveolar bone homeostasis.

Gingival crevicular fluid (GCF), serum, and saliva have been used routinely for detection of various markers of periodontal disease but in children saliva is best preferred procedure for sampling as it's much easier and more bearable for the patient. In addition, whole saliva represents a pooled sample with contributions from all periodontal sites, and analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site specific GCF analysis. [11] Not many studies are there in the recent past to evaluate the alkaline phosphatase levels in children less than 18 years of age. With this background the aim of our study was to assess whether salivary alkaline phosphatase levels can be a noninvasive marker for early inflammatory periodontal disease in children with uncontrolled type 1 diabetes mellitus.

MATERIALS AND METHOD

30 patients between the age group of 12- 18 years were selected for the study. Out of which 20 were diagnosed as type 1 diabetes mellitus. Three groups were formed; group 1 non diabetic patients, group 2 patients diagnosed as type 1 diabetes mellitus a year back and group 3 children diagnosed as type 1 diabetes mellitus for greater than 4 years. The diabetic patients were recruited for the study from various diabetic centers across Bangalore from January 2015 to March 2015. All subjects were matched for age, gender and socioeconomic status. Patient was explained the detail of the study and a written informed consent for volunteering to participate in the study was obtained. If minor the parents were explained and consent obtained for the same. The study was approved by the ethics committee of Bangalore Institute of Dental Sciences and Post Graduate Research Center. We used the following inclusion criteria: (i) diagnosis of Type 1DM 12 months prior to the study; (ii) presence of at least 20 teeth; Exclusion criteria were as follows: (i) presence of any important disease except for DM; (ii) smoking; (iii) having taken antibiotics, corticosteroids or nonsteroidal anti-inflammatory drugs within the 6 months prior to examination; and (iv) having undergone periodontal treatment within the previous 2 years.

Both blood and salivary samples were assessed after overnight fast; this was followed by periodontal examination on the same day.

Glycemic control: fasting venous blood was used to evaluate the glycosylated hemoglobin levels.

Periodontal examination: All subjects were subjected to periodontal examination by a single skilled periodontist (s). The examiner was unaware of the diabetic status of the individual. Plaque index, gingival index, gingival bleeding index and probing pocket depth were recorded using university of North Carolina (UNC) 15 probe,(Hu Friedy, Chicago, IL,USA) full mouth recording were taken at six sites per tooth; mesial, midbuccal, distal, on the buccal and lingual/palatal sites respectively. The mean scores of all the values were tabulated and sent for statistical evaluation.

Salivary collection: whole saliva was collected after overnight fasting by drooling method. The saliva samples were stored at − 20°C until analyzed for the salivary ALP activity. Before analysis, the collected saliva was centrifuged at 3,000 g for 10 minutes. The supernatant of saliva were used for analysis. The alkaline phosphatase activity was measured following DEA-AMP method in automated biochemistry analyzer spectophotometrically at 405 nm. The salivary ALP activity for each subject was expressed in U/L. Buffers used were DEA- diethanolamine, AMP-2-amino-2-methyl-1-propanol.

Statistical analysis

Data were entered using the Statistical Package for Social Sciences (SPSS version 20/PC; SPSS, Chicago, IL, USA). Level of significance was set at ≤ 0.05.

Kruskal Wallis ANOVA was done for comparison between three groups, Mann Whitney U test was done for comparison between two groups and Spearmans rank correlation method for correlating between the groups.

RESULTS

The mean age of the patients in all three groups ranged from 14 years to 16 years and there were no statistical difference between the groups with respect to age when compared by Mann Whitney U test. The mean plaque scores were 0.56 ± 0.31, 0.51 ± 0.24, and 0.72 ± 0.45 for the groups 1, 2 and 3 respectively. Intergroup comparison by Kruskal Wallis ANOVA (Table 1) and pair wise comparison by Mann Whitney U test showed no statistical differences between the groups (Table 2). The mean gingival index scores were 0.35 ± 0.24, 0.40 ± 0.22, and 0.69 ± 0.34 for the groups 1, 2 and 3 respectively. Intergroup comparison by Kruskal Wallis ANOVA (Table 1) and pair wise comparison by Mann Whitney U test showed significant statistical differences between the group1 and group 3 and between group 2 and group 3 at $p<0.05$ (Table 2). The mean PPD scores were 2.41 ± 0.14, 2.50 ± 0.10, and 2.64 ± 0.16 for the groups 1, 2 and 3 respectively. Intergroup comparison by Kruskal Wallis ANOVA Table 1) and pair wise comparison by Mann Whitney U test showed significant statistical differences between the group1 and group 3 and between group 2 and group 3 at $p<0.05$ (Table 2). The mean

Table 1: Statistical analysis of differences in Age, Periodontal parameters, and Biochemical parameters between the three groups by Kruskal Wallis ANOVA

GROUP	AGE	gender		PI (MEAN±SD)	GI (MEAN±SD)	PPD (MEAN±SD)	ALP (MEAN±SD)	HBA1C (MEAN±SD)
		M	F					
1 (N=10)	15.20±1.81	4	6	0.56±0.31	0.35±0.24*	2.41±0.14*	55.43±32.26*	-
2 (N=10)	14.60±2.12	5	5	0.51±0.24	0.40±0.22†	2.50±0.10†	73.27±20.78†	10.27±1.71
3 (N=10)	15.40±2.01	5	5	0.72±0.45	0.69±0.34	2.64±0.16	164.95±61.09	11.04±2.60

salivary ALP levels were 55.43 ± 32.26, 73.27 ± 20.78, and 164.95 ± 61.09 for the groups 1, 2 and 3 respectively. Intergroup comparison by Kruskal Wallis ANOVA (Table 1) and pair wise comparison by Mann Whitney U test showed significant statistical differences between the group1 and group 3 and between group 2 and group 3 at $p<0.05$ (Table 2).

Spearmans correlation between levels of salivary ALP to periodontal parameters showed positive correlation with GI, PPD in group 2 and in group 3 all the periodontal parameters positively correlated with increasing levels of ALP (Table 3, 4 and 5). No significant correlation could be seen between the diabetic group ALP levels and the glycosylated hemoglobin values. This could be since we have taken uncontrolled diabetes subjects for both the groups.

Table 2:Statistical analysis of pair wise comparisons in between the three groups by Mann-Whitney U test.

Parameters	Group 1 and Group 2	Group 1 and Group 3	Group 2 and Group 3
PI	p=0.7913	p=0.4057	p=0.3075
GI	p=0.5205	p=0.0126*	p=0.0376*
PPD	p=0.1306	p=0.0058*	p=0.0413*
ALP	p=0.0821	p=0.0005*	p=0.0007*

*p<0.05

Correlation of ALP in various groups

Table 3: Correlation between ALP levels with GI, PI and PPD by Spearman's rank correlation method in Group 1

Group 1	Correlation between ALP levels with			
	N	Spearman R	t-value	p-level
PI	10	0.5583	1.9033	0.0935
GI	10	0.9086	6.1512	0.0003*
PPD	10	0.9086	6.1512	0.0003*

*p<0.05

Table 4: Correlation between ALP levels with GI, PI, PPD, and HBA1c by Spearman's rank correlation method in Group 2

Group 2	Correlation between ALP levels with			
	N	Spearman R	t-value	p-level
PI	10	0.7866	3.6032	0.0069
GI	10	0.9879	18.0003	0.00001*
PPD	10	0.9999	36.9217	0.00001*
HBA1c	10	0.1945	0.5609	0.5902

*p<0.05

DISCUSSION

Strong evidence supports bidirectional relationship between diabetes mellitus and chronic perodontitis with many similarities in the pathobiology with exaggerated immune-inflammatory response being a critical player (Mealey and Oates 1996)[1]. Most of the studies link type 2 DM to chronic periodontits. Since both these conditions are prevalent largely in the age group of above 35 years of age linking these two conditions however have resulted in multiple research in this field. India being considered the diabetic capital of the globe has seen a rise in number of cases owing to the Asian Indian phenotype. Very few studies are there in literature comparing type 1 DM and chronic periodontits and previous studies studying this association in type 1 DM have wider age group including adults[12, 17]. The older assumption that periodontitis is a disease of aging is no longer tenable. The current view sees the greater periodontal destruction in the elderly as reflecting lifetime disease accumulation rather than an age-specific condition. The most rapid disease progression is seen in that relatively small number of persons in whom the disease starts young, and there is some evidence that these individuals have some genetic predisposition to periodontitis[13]. Studies have concluded that in children with diabetes, periodontits begins at puberty and progresses with age [12, 14]. In a large cohort of young patients with diabetes, Lalla *et al* [15, 16] showed that periodontal destruction starts early in life and becomes more prominent as children become adolescents. It is also important to examine the relationship between the time elapsed from diabetes mellitus diagnosis and periodontal disease, since some authors have reported a correlation between a longer duration of DM and increased severity of periodontal disease [17].Recently concluded Cochrane review echoed the fact in their conclusion that there is little data regarding type 1 diabetes glycemic

Table 5: Correlation between ALP levels with GI, PI, PPD, and HBA1c by Spearman's rank correlation method in Group 3

Group 3	Correlation between ALP levels with			
	N	Spearman R	t-value	p-level
PI	10	0.6565	2.4619	0.0392*
GI	10	0.8511	4.5846	0.0018*
PPD	10	0.7477	3.1850	0.0129*
HBA1c	10	0.3951	1.2166	0.2584

*p<0.05

control.[18] Hence with this background the primary objective of our study was to assess the possible correlation existing between periodontal disease, type 1 DM, and its duration to salivary ALP levels in children.

30 patients completed the study protocol, the study design included recording of important clinical periodontal parameters such as PI, GI, and PPD, and glycosylated hemoglobin and correlated each of these to the duration of patients with type 1DM. Strength in our study is full mouth examination and patients with even single localized periodontal pocket were taken into consideration and division of the diabetic patients based on duration,

In our study the mean age and plaque score did not show any difference at baseline, in spite of this being same; the gingival index showed statistical significant difference between the healthy children and the children with type 1 diabetes mellitus. It was also noted that the type 1 diabetes for longer duration showed greater gingival index than the diabetic group of less than four years. This supported the fact that longer the duration of diabetes mellitus longer in the destruction of the periodontium as echoed by studies that show that plaque index gingival index and PPD increase with the duration of diabetes.[19] The group of subgingival organisms found in periodontal lesions from patients with Type 1 DM differs quantitatively from that of Adult Periodontitis and localized juvenile periodontitis/ aggressive periodontitis, and represents another major constellation of bacteria associated with periodontal bone loss. In our study ALP is also noticed to be increased in group 4 than in group 2 and group 1. Intergroup comparison showed alkaline phosphatase is seen more in type 1 diabetes mellitus of longer duration than recently diagnosed type 1 DM; where as in systemically healthy children group it was least. This could be explained by the fact that in the periodontium, ALP is very important enzyme as it is part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. [20] ALP is considered to be an important indicator of osteoblastic activity. ALP is detected in the parotid, submandibular, and minor salivary glands, as well as in desquamated epithelial cells, leukocytes, and bacteria from dental plaque. The presence of ALP in the saliva is usually indicative of inflammation and destruction of the periodontal tissues. The level of ALP is positively correlated with the severity of the periodontal disease.[11]Some studies have shown a remarkably increased activity of ALP in the acute phase of periodontal disease, and after periodontal therapy, the activities of these enzymes restored to the values found in healthy persons.[21, 22]

GCF, Saliva and Serum have been used in periodontal research, but in children it would be always easier if saliva could be used as a reliable tool. In healthy persons, their activities are within normal level. In periodontitis the cells become damaged, due to edema or destruction of a cellular membrane, as a result of which there is an increased release into the gingival crevicular fluid and saliva where their activity can be measured. Due to this, these enzymes can be biochemical markers of the functional condition of periodontal tissues.[23] Previous studies have mainly investigated the activities of these enzymes in gingival crevicular fluid, which has a much closer contact with the periodontal tissues, and due to this, it surely reflects the occurrences in them much better. However, the problem with the gingival crevicular fluid is that the technique of collecting it is rather complicated, and as a routine procedure, which possibly may be established, it will be hardly feasible in practice for young children[24]. However, the evidence for an association between type 1 DM and periodontitis is insufficient, Pooled differences of clinical measures of periodontal disease did not differ significantly between type 1 diabetics and non-diabetics, and longitudinal studies showed conflicting findings. The lack of association between type 1 DM and periodontal disease can be explained by the low mean age of the subjects, namely, between 11and 15 years. Even diabetic subjects in this age range do not frequently develop destructive periodontal disease. Hence in this study we included all the patients who have gingivitis as well as localized/generalized periodontits and full mouth periodontal clinical parameters which were recorded so that even a slight change in the clinical parameters would be reflected in the ALP levels and studies have proven ALP to be a good indicator of periodontal disease in both acute and chronic forms[8], interventional studies have also shown that the activity of ALP restored to the value as found with the healthy persons,[25] ALP could fairly predict the disease activity and this study proves that it could be used as an important parameter to assess in children with type1 diabetes mellitus for predicting their susceptibility to more destructive forms of periodontitis at a very early stage of the disease. In addition, whole saliva represents a pooled sample with contributions from all periodontal sites, and analysis of biomarkers in saliva may provide an overall assessment of disease status as opposed to site specific GCF analysis.

CONCLUSIONS

Thus within the limitation of this study it can be concluded that salivary alkaline phosphatase level could be used as early marker for periodontal disease in children with uncontrolled type 1 diabetes mellitus and longer the duration of diabetes more is the risk for periodontal disease.

Interventional studies with a larger sample size and comparison between the serum, salivary and GCF alkaline phosphatase levels would be the next step in our research after this pilot study.

REFERENCES

1. Mealey BL, Oates TW. American Academy of Periodontology. Diabetes mellitus and periodontal diseases. J Periodontol 77: 1289–1303, 2006.
2. Nathan DM, Cleary PA, Backlund JY et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 353:2643–2653, 2005.
3. Iacopino AM. Periodontitis and diabetes interrelationships: role of inflammation. Ann Periodontol 6:125–137, 2001.
4. Hugoson A, Thorstensson H, Falk H, Kuylenstierna J. Periodontal conditions in insulin-dependent diabetics. J Clin Periodontol 16: 215–223, 1989.
5. Mealey BL, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. CurrOpin Endocrinol Diabetes Obes 15(2): 35–141, 2008.
6. Taylor GW, Borgnakke WS. Periodontal disease: associations with diabetes, glycemic control and complications. Oral Dis 14: 191–203, 2008.
7. Aspriello SD, Zizzi A, Tirabassi G, Buldreghini E, Biscotti T, Faloia E, Stramazzotti D, Boscaro M, Piemontese M. Diabetes mellitus-associated periodontitis: differences between type 1 and type 2 diabetes mellitus. J Periodont Res 46: 164–169, 2011.
8. McCauley LK, Nohutcu RM. Mediators of periodontal osseous destruction and remodeling: Principles and implications for diagnosis and therapy. J Periodontol 73(11): 1377-91, 2002.
9. Lisgarten MA. Periodontal probing: What does it mean? J ClinPeriodontol 7: 165-76, 1980.
10. Reddy MS. The use of periodontal probes and radiographs in clinical trials of diagnostic tests. Ann Periodontol 2: 113-122, 1997.
11. Numabe Y, Hisano A, Kamoi K, Yoshie H. Periodontology Analysis of saliva for periodontal diagnosis and monitoring. Dent Jpn 40: 115- 27, 2004.
12. Burt BA. Periodontitis and aging: Reviewing recent evidence. *J Am Dent Assoc* 125: 273-279, 1994.
13. Parkhill JM, Hennig BJ, Chapple IL, Heasman PA, Taylor JJ. Association of interleukin-1 gene polymorphisms with early-onset periodontitis. J Clin Periodontol 27: 682-689, 2000.
14. Manouchehr-Pour M, Bissada NF. Periodontal disease in juvenile and adult diabetic patients: A review of the literature. J Am Dent Assoc 107: 766-770, 1983.
15. Lalla E, Cheng B, Lal S, et al. Periodontal changes in children and adolescents with diabetes: A case-control study. Diabetes Care 29: 295-299, 2006.
16. Lalla E, Cheng B, Lal S, et al. Diabetes mellitus promotes periodontal destruction in children. J Clin Periodontol 2007; 34:294-298
17. Firatli E, Yilmaz O, Onan U. The relationship between clinical attachment loss and the duration of insulin-dependent diabetes mellitus (IDDM) in children and adolescents. J Clin Periodontol 23: 362-6, 1996.
18. Simpson TC, Needleman I, Wild SH, Moles DR, Mills EJ. Treatment of periodontal disease for glycemic control in people with diabetes. *Cochrane Database of Systematic Reviews* 2010, Issue 5. Art. No.: CD004714. DOI: 10.1002/14651858.CD004714.pub2.
19. Trevenon. T, Knuuttila. M relationship of diabetes control on periodontal pocketing and alveolar bone level. Oral sur oral pathol oral radiolendo 61: 346-49, 1981.
20. Yan F. Alkaline phosphatase level in gingival crevical fluid of periodontitis before and after periodontal treatment. Chinese Journal of Stomatology 30: 255–66, 1995.
21. Chapple IL, Socransky SS, Dibart S, Glenwright IL, Mathews JB. Chemiluminescent assay to alkaline phosphatases in human gingival crevical fluid: Investigations with on experimental gingivitis model and studies on the source of the enzyme within crevical fluid. J Clin Periodontol 23: 587–94, 1996.
22. Nakashima K, Roechrich N, Cimasoni G. Osteocalcin, prostaglandin E2 and alkaline phosphatases in gingival crevical fluid: Their relation to periodontal status. J Clin Periodontol 21: 327–33, 1994.
23. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis- a review. J Clin Periodontal 27: 453-65, 2000.
24. Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 11:821–78, 1996.
25. Shu L, Guan SM, Fu SM, Guo T, Cao M, Ding Y. Estrogen Modulates Cytokine Expression in Human Periodontal Ligament Cells. J Dent Res 87: 142-7, 2008