RESEARCH ARTICLE

Serum enzyme levels in patients of Diabetes mellitus with periodontitis

[1]Santosh Gawali, [2]Chavan P R, [3]Deepak A D

[1]Senior Resident, [2]Professor, [3]Professor and HOD, Department of Biochemistry, MGM Medical College, Kamothe, Navi Mumbai, Maharashtra, India

Correspondence to: Dr. Santosh Gawali **(santoshgawali27@yahoo.in)**

Abstract

Diabetes mellitus is a risk factor for development of periodontal disease with progressive periodontal destruction seen in diabetic patients. Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers. The aim of this study was to identify a simple chair-side test as a marker for diagnosis of periodontitis in patients with Diabetes mellitus. Levels of AST, ALP, LDH and β Glucuronidase in serum of healthy subjects, patients of Diabetes mellitus and Periodontitis with and without Diabetes were estimated and compared with clinical parameters like Probing pocket depth, Plaque Index and Gingival Index. All enzymes were maximally raised in diabetic cases with periodontitis. Serum Glucuronidase correlated significantly with PPD, PI and GI.

Of all the enzymes in serum, β-glucuronidase levels correlated best with the clinical indices and may be employed on routine basis as a chair side test for screening and diagnosis of patients with periodontitis in diabetics.

Keywords: Type-2 Diabetes mellitus, Periodontitis, biomarkers, β Glucuronidase

Introduction

Diabetes affects a huge population worldwide and has reached epidemic status. As per the data provided by International Diabetes Federation, the worldwide prevalence of Diabetes in 2011 was 366 million and this number is projected to reach 552 million by 2030. [1] Long-term diabetic oral complications include microvascular diseases like Xerostomia, greater susceptibility of oral tissues to trauma and more opportunistic infections (e.g., candidiasis). Diabetics are prone for greater accumulation of plaque and risk of caries with increased susceptibility to periodontal disease and development of periodontal abscesses. Peripheral neuropathy is a significant oral complication causing oral paraesthesia, burning mouth or tongue, altered taste sensations etc. [2]

The association between periodontal disease and diabetes has been explored in several studies over the years, and it is generally accepted that periodontal disease is more prevalent and more severe in persons with

diabetes than in nondiabetic persons. Indeed, the periodontal signs and symptoms are now recognized as the "sixth complication" of diabetes. [3] Since periodontitis is readily modifiable risk factor, major efforts should be directed at preventing periodontitis in patients who are at risk of diabetes. [4]

Conventional methods for diagnosing periodontal disease are measurement of probing depth and clinical attachment loss by the periodontal probe along with gingival index, plaque index and radiographic evaluations of alveolar bone loss. These methods measure the damage from the past episodes of destruction. [5] There is a need for the development of new diagnostic tests that can detect the presence of active disease, predict future disease progression, and evaluate the response to periodontal therapy, thereby improving the clinical management of periodontal patients. Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified by objective measures such as biomarkers. [6] Biochemical analysis may include detection of specific inflammatory markers, proteolytic and hydrolytic enzymes, cell death and connective tissue degradation products and bone-related biomarkers.

The purpose of the study was to identify enzymatic biomarkers of periodontal tissue destruction and to correlate their levels with the severity of periodontal disease in diabetic patients, so that such a biomarker may be used as a screening tool for periodontitis in every diabetic case. The aim of this study was to identify a simple chair-side test as a marker for diagnosis of periodontitis in patients with Diabetes mellitus. For this we estimated and compared the levels of AST, ALP, LDH and β Glucuronidase in serum of healthy subjects, patients of Diabetes mellitus and Periodontitis with and without Diabetes. Suitability of these enzymes as a biomarker of periodontitis was assessed in patients of diabetes by studying their association with clinical indices.

Materials and methods:

The study was conducted at the Department of Biochemistry, MGM Medical College Navi Mumbai and Department of Periodontology, MGM Dental College Navi Mumbai. The study was ethically approved by Institutional Review Board and written informed consent was obtained from all the participants prior to the study.

A total of 40 subjects were divided into four groups as: Group-I Healthy individuals (n=10), Group-II Type-2 Diabetes without Periodontitis (n=10), Group-III Periodontitis without Type-2 Diabetes (n=10) and Group-IV Type-2 Diabetes with Periodontitis (n=10).

Patients selected for the study had Type-2 Diabetes mellitus for a period of at least 3 years and were on diabetic treatment without any other systemic diseases or diabetic complications like neuropathy, nephropathy, retinopathy etc. Those patients diagnosed clinically as having periodontitis and not undergone any periodontal treatment since last one year were included. Patients less than 30yrs, smokers and ex-smokers or those having history of systemic illness and high liver enzyme activities were excluded. The periodontal involvement was confirmed when presence of hard deposits or inflammation or

bleeding on probing was observed by the attending periodontologist.

Clinical parameters: The relevant clinical history was recorded for all the patients. A careful oral examination was carried out with the help of mouth mirror and graduated periodontal probe and following clinical parameters were recorded.

Probing Pocket Depth (PPD in mm) was measured with a William's graduated periodontal probe held parallel to the vertical axis of the tooth and walking it circumferentially around each surface of each tooth. The area of deepest penetration was recorded for each individual tooth. Plaque Index (PI) was assessed by Sillness and Loe, 1967 scoring criteria. The score for PI ranged from 0 to 3 for control and study group. Gingival Index (GI) was assessed by Sillness and Loe, 1963 scoring criteria. The severity of gingival inflammation this criteria ranges from 0.1 to 3.0

Sample collection and preparation:

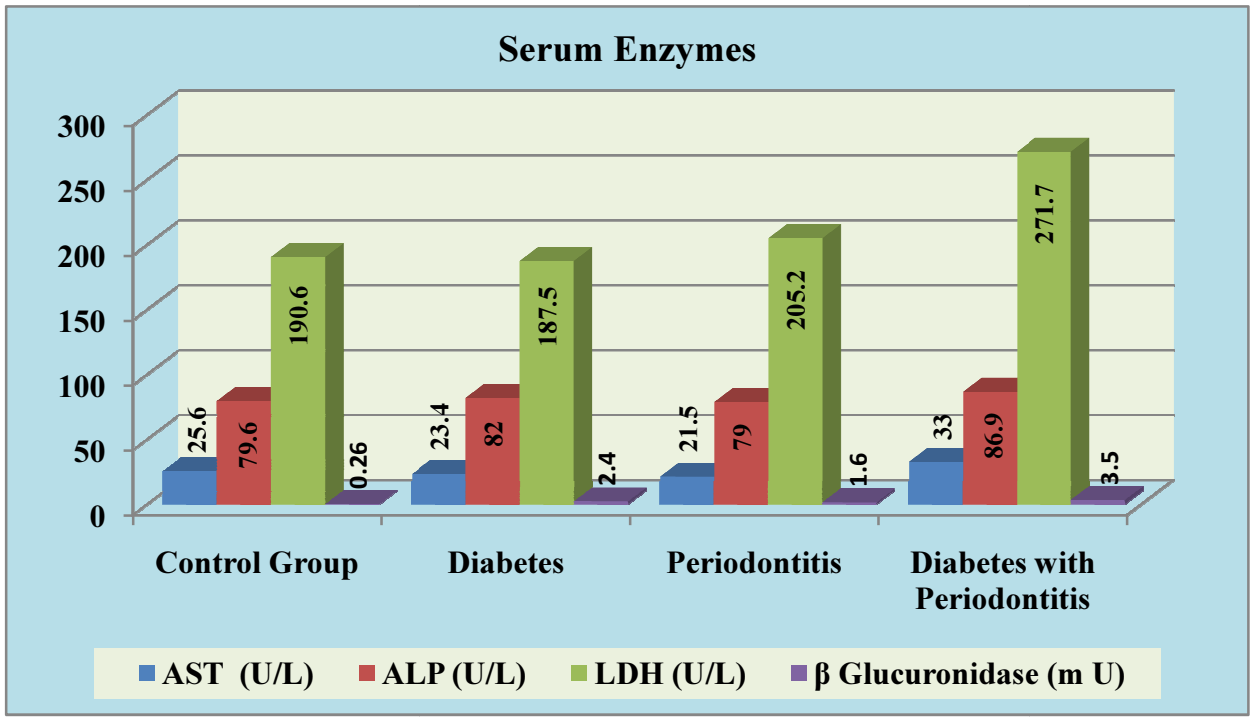
Fasting venous blood was collected under strict aseptic conditions for obtaining serum which was used for enzyme assays.

Enzyme assay – Aspartate transaminases levels were analyzed by IFCC method without P-5-P. Alkaline phosphatase was assayed by IFCC -AMP Kinetic method. Lactate dehydrogenase levels were estimated by IFCC method. All these enzymatic assays were performed on Beckman – Coulter AU 480 plus autoanalyser. β Glucuronidase activity was determined by the method of Fishman et al using phenolphthalein glucuronide as substrate. [7, 8]

Results:

Table 1: Serum Enzymes (Mean ± S.D.) in Control and Study Groups

Parameter	**Group I** (Control)	**Group II** (Diabetes)	**Group III** (Periodontitis)	**Group IV** (Diabetes with Periodontitis)
AST (U/L)	25.6 ± 8.5	23.4 ± 7.5	21.5 ± 8	33 ± 7.7
ALP (U/L)	79.6 ± 15.9	82 ± 16.9	79 ± 21	86.9 ± 15.2
LDH (U/L)	190.6 ± 26.4	187.5 ± 32.2	205.2 ± 44.4	271.7 ± 49.9
β Glucuronidase (m U)	0.26 ± 0.08	2.4 ± 1	1.6 ± 1.1	3.5 ± 0.4



All Serum Enzymes were raised in group III (periodontitis) and group IV (diabetes with periodontitis). The levels were highest in group IV.

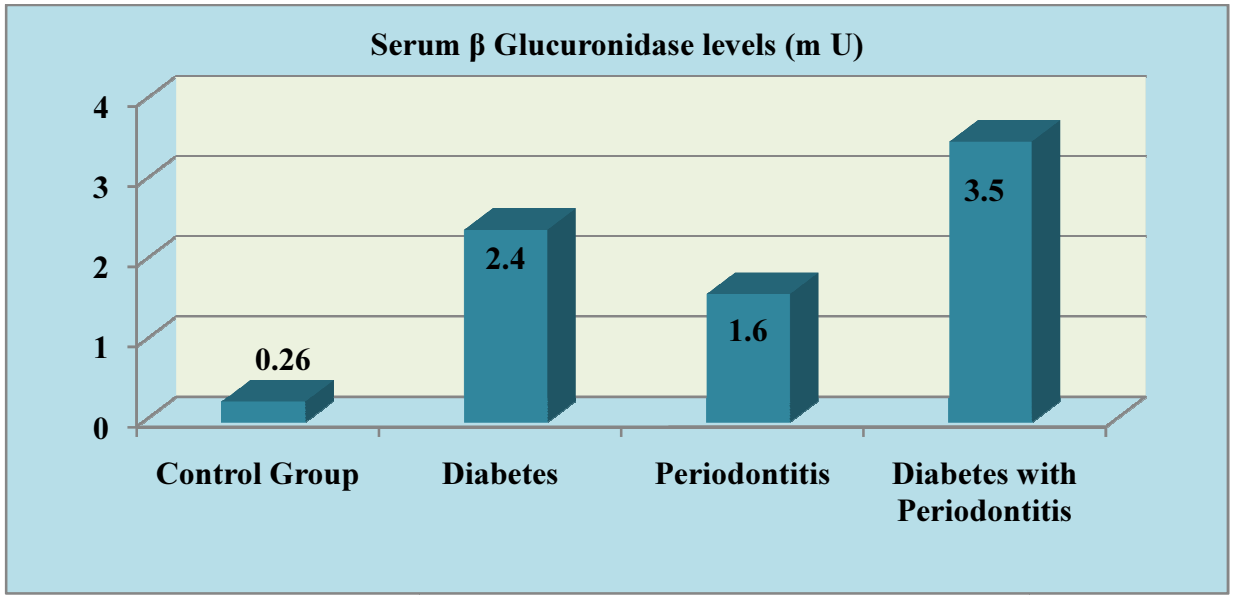


Table No.2: Intergroup comparison of serum enzyme levels *(Using Tuckey's POST HOC Test)*

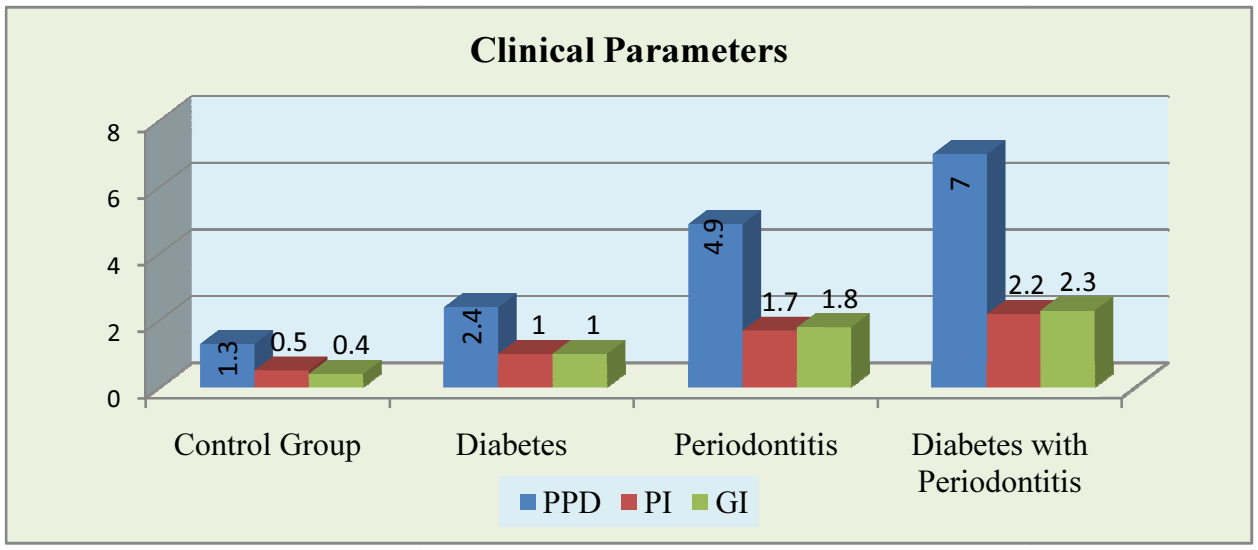
Parameter	Group	Group	P-value
AST	Control Group	Diabetes	0.928
		Periodontitis	0.663
		Diabetes with Periodontitis	0.175
	Diabetes	Periodontitis	0.949
		Diabetes with Periodontitis	0.049*
	Periodontitis	Diabetes with Periodontitis	0.013*
ALP	Control Group	Diabetes	0.976
		Periodontitis	1.000
		Diabetes with Periodontitis	0.786
	Diabetes	Periodontitis	0.961
		Diabetes with Periodontitis	0.952
	Periodontitis	Diabetes with Periodontitis	0.743
LDH	Control Group	Diabetes	0.998
		Periodontitis	0.840
		Diabetes with Periodontitis	< 0.010**
	Diabetes	Periodontitis	0.748
		Diabetes with Periodontitis	< 0.010**
	Periodontitis	Diabetes with Periodontitis	0.003**
β Glucuronidase	Control Group	Diabetes	< 0.010**
		Periodontitis	0.002**
		Diabetes with Periodontitis	< 0.010**
	Diabetes	Periodontitis	0.160
		Diabetes with Periodontitis	0.023*
	Periodontitis	Diabetes with Periodontitis	< 0.010**

: p value ≤ 0.05: fairly significant; **: p value ≤ 0.01: highly significant

Serum AST levels showed fairly significant increase when group II was compared with group IV (p=0.049). When group III was compared to group IV, the AST levels were increased significantly (p= 0.013). Serum ALP levels did not show any significant increase in any of the groups. With regards to serum LDH levels, significant increase was found in group IV when compared to all other three groups (p < 0.01). Serum β Glucuronidase levels in diabetics did not differ statistically from periodontitis group. While the levels showed statistically significant differences in rest all the groups.

Table No. 3: Clinical Parameters (Mean ± S.D.) in Control and Study Groups

Parameter	Group I (Control)	Group II (Diabetes)	Group III (Periodontitis)	Group IV (Diabetes with Periodontitis)
PPD	1.3 ± 0.4	2.4 ± 0.5	4.9 ± 0.8	7 ± 0.8
PI	0.5 ± 0.2	1 ± 0.3	1.7 ± 0.2	2.2 ± 0.3
GI	0.4 ± 0.1	1 ± 0.3	1.8 ± 0.2	2.3 ± 0.3

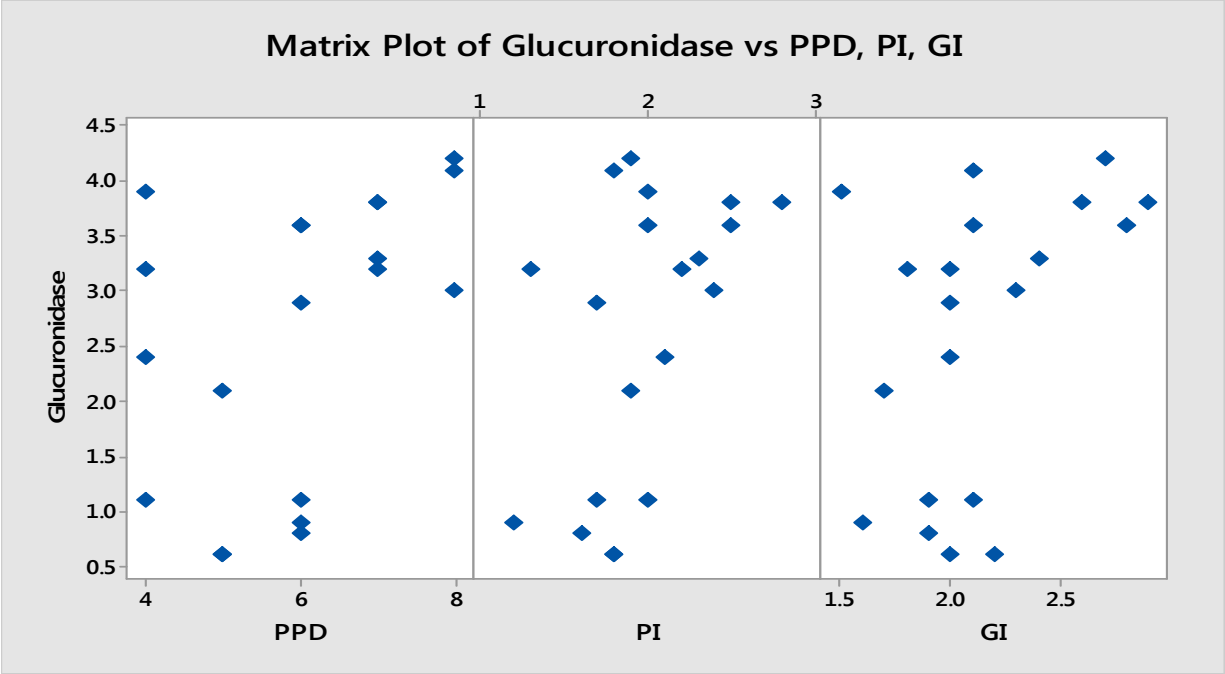


All Clinical Parameters were raised in group II (diabetes), group III (periodontitis) and group IV (diabetes with periodontitis). The levels were highest in group IV.

Table No. 4: Correlation between Clinical Parameters and serum Enzymes

	PPD		PI		GI	
Parameter	**CC**	**p-value**	**CC**	**p-value**	**CC**	**p-value**
AST	0.436	0.05*	0.349	0.13	0.246	0.29
ALP	-0.178	0.45	0.13	0.58	0.02	0.93
LDH	0.509	0.02*	0.308	0.19	0.377	0.10
Glucuronidase	0.445	0.05*	0.503*	0.02*	0.44	0.05*

CC: Correlation Coefficient; ***:** p value ≤ 0.05: fairly significant ****:** p value ≤ 0.01: highly significant



Serum LDH and AST correlated significantly with PPD. Serum Glucuronidase correlated significantly with PPD (Correlation Coefficient 0.445, p = 0.05) with PI (Correlation Coefficient 0.503, p = 0.02) and also with GI (Correlation Coefficient 0.44, p = 0.05)

DISCUSSION

Diabetes mellitus is a risk factor for development of periodontal disease with progressive periodontal destruction seen in diabetic patients. Periodontal diseases are "silent" in nature; most patients do not realize the presence of disease until significant destruction has occurred. [9] Hence accurate detection of periodontal sites exhibiting disease progression or those at risk for future deterioration has always proven difficult. [10]. Traditional diagnostic procedures are inherently limited, in that only disease history, not current disease status, can be assessed. These conventional disease diagnosis techniques lack the capacity to identify highly susceptible patients who are at risk for future breakdown. Therefore we have attempted to study the levels of AST, ALT, LDH and β Glucuronidase in serum and correlated them with clinical parameters, in order to establish a suitable biomarker for periodontitis

Aspartate aminotransferase (AST)

Aspartate aminotransferase is a tissue destruction biomarker released from necrotic cells in gingival fluid and is associated with periodontitis severity. [6] It has proven to be a strong diagnostic indicator of periodontal inflammatory lesions as it is one among the enzymes that is most commonly associated with tissue damage. AST is released during periodontal tissue destruction process and passes with the inflammatory exudate.

In our study increase in serum AST levels, was observed only in group IV i.e. diabetes with periodontitis (table no.1). When serum AST levels of all four groups were compared, slight increase was noted only when group II was compared with group IV as well as when group III was compared with group IV ($p < 0.05$) as can be seen from table no.2. Serum AST correlate fairly with PPD ($P= 0.05$).

Oringer et al studied the relationship between gingival fluid aspartate aminotransferase levels and periodontal disease progression and concluded that AST as a biomarker does not discriminate between progressive sites and sites that are stable but inflamed. [6]. Barbosa et al found significant correlation in sites with periodontal disease and AST levels in patients with chronic periodontitis on evaluation of 147 sites in 22 patients. They noted a high incidence of gingival inflammation with higher AST scores, and that this incidence increased with greater inflammation. [11]

Alkaline phosphatase (ALP)

Alkaline Phosphatase is a membrane bound glycoprotein produced by many cells within the area of periodontium and gingival crevice. [12] It is released from polymorphonuclear neutrophils during inflammation, from osteoblasts during bone formation and from periodontal ligament as well as fibroblasts during periodontal regeneration. [12] In the periodontium, ALP is a very important enzyme as it is a part of normal turnover of periodontal ligament, root cementum and maintenance, and bone homeostasis. [13] High ALP activity reflects destruction and repair of connective gingival tissue during the chronic inflammation of periodontal disease. [14]

In our study, we found that there was no significant increase in serum ALP levels in the study groups when compared to control group as seen in table no. 1 and 2. However, we found no correlation between clinical parameters and serum ALP levels.

Gilbert et al. (2003) studied ALP activity in serum from patients with chronic periodontitis and showed a relationship between loss of attachment in periodontal disease and ALP activity in serum. [13]. Gao et al. (1999) found that ALP activity was highest in osteoblasts, moderate in periodontal ligament fibroblasts, and lowest in gingival fibroblasts. [13] Ishikawa and Cimasoni in 1970 quantified ALP in gingival fluid demonstrating that, the levels of enzyme in gingival fluid are three times greater than those in serum. They showed that periodontal bone is also a possible source of ALP and is indeed highly active in this tissue [12]

Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an intracytoplasmic enzyme which may contribute to the progression of periodontal disease. [10] LDH increases when cell death begins, reaches a maximum after some time, and then begins to decline

With regards to serum LDH levels, of our study increased levels were seen both in Group III and Group IV.(table no.1) When intergroup comparisons were done it was noted that there was significant increase in LDH levels in patients of Group IV when

compared with rest of three groups ($p < 0.01$) as can be seen from table no.2. Our results are in accordance with the study by Ebele who suggested that rise in LDH is an indicator of higher level of cellular damage. [15] Nomura and Tamaki [16] concluded that the sensitivity and specificity of saliva LDH for screening periodontitis were greater than 0.65 as diagnosed by probing depth. In our study serum LDH levels correlated significantly with PPD (Correlation Coefficient - 0.509, $p = 0.022$)

Beta glucuronidase

β-glucuronidase is a lysosomal acid hydrolase enzyme which is capable of breaking down connective tissue ground substances. It has been used as a potential marker for periodontal disease activity. This acid glycohydrolase is used as a marker for primary granule release from polymorphonuclear leukocytes (PMN) [17]

During active phase of periodontal disease there is influx of PMN cells into the gingival crevice. Six fold increase in β-glucuronidase has been found in periodontal diseases. [17] The activity of β-Glucuronidase is associated with severity of inflammation and also with the presence of putative pathogenic flora.

In our study the serum β Glucuronidase levels of all the groups were increased significantly compared to controls as seen from table no.1. This enzyme was noted to be highest in patients of group IV. The diabetic subjects did not differ statistically in their serum enzyme level from periodontitis group. While the levels showed statistically significant differences in all the rest of groups (table no.2) Serum β-glucuronidase levels correlated significantly with all clinical indices; PPD ($p= 0.05$), with PI ($p = 0.024$) and with GI ($p= 0.05$) as seen in table no.4

Aarti et al [18] demonstrated that there was higher level of gingival fluid glucuronidase activity in patients of diabetes with chronic periodontitis as compared to non-diabetic with chronic periodontitis. Serum β-glucuronidase has not been studied as a marker enzyme and thus not reported in literature. In our study we found this enzyme to be the biomarker to identify patients of periodontitis having diabetes mellitus as it correlates significantly with the clinical indices.

CONCLUSION

Of all the enzymes in serum, β-glucuronidase levels correlated best with the clinical indices. We suggest that serum β-glucuronidase may be employed on routine basis as a chair side test for screening and diagnosis of patients with periodontitis in the population of diabetics as it is simple, cheap and has less turn around time.

REFERENCES

[1]. Abhijit N. Gurav. Periodontitis and Insulin Resistance: Casual or Causal Relationship? Diabetes Metab J. 2012; 36:404-41.

[2]. Debora c. Matthews. The Two-Way Relationship between Diabetes and Periodontal Disease. Supplement to RCDSO DISPATCH Fall. 2005.

[3]. Harald loe. Periodontal Disease, The sixth complication of diabetes mellitus. Diabetes care. 1993; 16(1).

[4]. Tara B. Taiyeb-Ali, Renukant P.Cheta Raman, Rathna D. Vaithilingam.

Relationship between periodontal disease and diabetes mellitus: an Asian perspective. Periodontology2000. 2011; 56: 258- 268.

[5]. Dabra S, Kamalpreet. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. J Indian Soc Periodontol. 2012; 16(3): 358–364.

[6]. Mario Taba, Janet Kinney, Amy S. Kim, William V, Giannobile. Diagnostic Biomarkers for Oral and Periodontal Diseases. Dent Clin North Am. 2005; 49(3): 551–6.

[7]. M. M. Khanolkar, A. V. Sirsat. Modified method for determination of serum beta-glucuronidase: A comparative study using P-nitrophenyl glucuronide and phenolphthalein glucuronide as substrate in gastrointestinal tract carcinomas. Indian Journal of Clinical Biochemistry. 1997;12(1): 67-70.

[8]. Gabor Szasz. Comparison between p-Nitrophenyl glucuronide Phenolphthalein Glucuronide as substrates in assay of glucuronidase. Clinical Chemistry. 1967;13 (9): 752-759.

[9]. Gaurav malik, Gurvanit lehl, Manjit talwar. Association of periodontitis with diabetes mellitus: a review. Journal a/Medical College Chandigarh. 2011; 1(1):10-14.

[10]. Myriam Koss, Castro. Enzymatic Profile of Gingival Crevicular Fluid in Association with Periodontal Status. Lab medicine. 2009; 40(5): 277-280.

[11]. Emílio Barbosa e Silva, Sergio Luis S. Salvador, Jose Carlos Fogo, Rosemary Adriana C. Marcantonio. Use of Aspartate aminotransferase in diagnosing periodontal disease: a comparative study of clinical and microbiological parameters. Journal of Oral Science. 2003; 45(1): 33-38.

[12]. Ranjan Malhotra, Vishakha Grover, Anoop Kapoor, Rupika Kapur. Alkaline phosphatase as a periodontal disease marker. Indian J Dent Res. 2010; 21(4):531-6.

[13]. S Desai, H Shinde, J Mudda, V Patil. Levels of Alkaline Phosphatase (ALP) In Saliva of Patients with Chronic Periodontitis; a Clinical and Biochemical Study. The Internet Journal of Dental Science. 2009; 8(1)

[14]. Caroline Perozini. Gingival crevicular fluid biochemical markers in periodontal disease: A cross-sectional study. Quintessence International. 2010; 41(10) 877-883.

[15]. Ebele J., Ikelpeazu, Emeka E., Neboh, Iggnatius C., Maduka,E.G., Anyanwu, N.S. Okenyi. Periodontal Disease and type 2 diabetes: effects on salivary enzyme activities. Int J Diabetes. 2011; 31(1): 9-13.

[16]. Yoshiaki Nomura, Yoh Tamaki. Screening of periodontitis with salivary enzyme tests. Journal of Oral Science. 2006; 48(4): 177-83.

[17]. Subrahmanyam MV, Sangeetha M. Gingival Crevicular Fluid A Marker of the Periodontal Disease Activity. Indian J Clin Biochem. 2003; 18:5-7.

[18]. .Aarti Chowdhary, G. V. Gayathri, and Dhoom Singh Mehta. Comparative analysis of GCF β-glucuronidase level in diabetic and nondiabetic patients with chronic periodontitis: A clinicobiochemical study. J Indian Soc Periodontol. 2008; 12(1): 16–20.

Site this article as: Santosh Gawali, Chavan P R, A D, Serum enzyme levels in patients of Diabetes mellitus with Periodontitis, Int J Medicine and Allied Health Sciences 2014; 1: 116-125