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Comparison of Salivary Beta Glucuronidase Activity in Chronic Periodontitis Patients with or Without Diabetes Mellitus-A Clinico Biochemical Study

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Abstract

Background and objectives: Periodontitis involve a complex interplay of micro-organisms and host immune response via numerous mediator molecules playing strategic roles in its pathogenesis. Diabetes promotes periodontitis through an exaggerated inflammatory response to the periodontal microflora and thereby causes delayed tissue recovering PMNs are a critical component of the local inflammatory response in the periodontal disease. β glucuronidase is an important component of primary granules of polymorphonuclear leukocytes (PMNs). Thus, the present study was conducted to estimate the beta glucuronidase activity in chronic periodontitis patients with and without diabetes mellitus.

Method: This study was conducted on 45 patients. The periodontal status was assessed by recording clinical parameters (Modified sulcular bleeding index, Probing depth, Clinical Attachment level). The patients were divided into 3 groups of 15 each namely, Group I: Chronic periodontitis patients with diabetes mellitus, Group II: Chronic periodontitis patients without diabetes, Group III: Patients with no signs of gingival inflammation (healthy group). Salivary Beta glucuronidase activity was estimated by spectrophotometer using phenolphthalein glucuronic acid, acetate buffer and 2-amino-2-methyl-1-propanol buffer. The statistical analysis was done using ANOVA and Tukeys test.

Interpretation and conclusion: The salivary beta glucuronidase activity showed statistically very high

difference between group I and group II. It also showed that there were significant correlations between the clinical parameters and beta glucuronidase activity. Thus suggesting that salivary beta glucuronidase activity was increased in chronic periodontitis with diabetes mellitus. Beta glucuronidase can be a potential biochemical marker for periodontal destruction.

Keywords: Beta glucuronidase, diabetes, periodontitis

Introduction

Periodontitis is a group of inflammatory diseases which affect the connective tissue attachment and supporting bone around the teeth whose initiation and progression depends on the presence of virulent microorganisms capable of causing disease.[1] This inflammatory disease is caused by a complex of anaerobic, Gram-negative bacteria. An imbalance between a localized infection and exaggerated host inflammatory response plays a vital role in determining damage to gingival tissue.[2]

Various biomarkers like saliva, GCF, serum are used for screening and predicting the early changes in the periodontal tissues and also to determine the efficacy of the treatment. Saliva is widely used as a biomarker to determine the periodontal disease activity because it allows rapid screening, provides accurate information, and enables reliable evaluation of periodontal disease condition.[3]

PMNs are a critical component of the local inflammatory response in the periodontal disease.

PMNs contain proteolytic and hydrolytic enzymes, that produce active oxygen metabolites which upon release can cause tissue destruction.[4] β glucuronidase is a neutrophil derived lysosomal acid hydrolase enzyme which is stored in the neutrophil primary (azurophil) granules and is released in response to inflammation during periodontal destruction. It is a potential indicator of neutrophil (PMNs) influx into the crevicular environment.[5] β Glucuronidase together with hyaluronidase, is involved in the catabolism of proteoglycans and non-collagenous matrix degradation.[6,7]

The association between diabetes and periodontal disease has received tremendous amount of evidential support over the years.[8] A number of proinflammatory cytokines produced in inflamed periodontal tissue, including tumor necrosis factor-α, interleukin 1 and 6, antagonize insulin. These mediators gain access to the circulation via periodontal microcirculation and can affect tissues and organs at distant sites.[9]

One hypothesis to explain damage in the periodontal tissues states that advanced

glycosylation end products (AGEs) that appear in diabetic patients due to the hyperglycemia make the immune system hyper- reactive to dental plaque, increasing the destruction of periodontal support.[10] The formation of AGEs occurs when excess available glucose is in contact with structural and other proteins. This process is not driven enzymatically, and once they are formed, AGEs bind to a specific cellular receptor, known as the receptor for AGE (RAGE). RAGE is found on endothelial cells and monocytes, which is of importance in periodontitis. The binding of AGE and RAGE causes a series of proinflammatory events that might be self-sustaining because AGE - RAGE binding on the surface of endothelial cells induces the expression of vascular cell adhesion molecule-1 that attracts monocytes to the luminal side of the endothelial cells, thus perpetuating the inflammatory response.[9]

The aim of this study is to estimate Salivary Beta Glucuronidase activity in Chronic Periodontitis with and without Diabetes Mellitus and healthy control group to compare the salivary Beta Glucuronidase activity and Clinical parameters within the groups.

Materials and Methods

Total of 45 subjects (both sexes) were selected from Outpatient department of Periodontics, A.J Institute of Dental Sciences, Mangalore between the age of 20- 60 years. Informed consent was taken from all the participants. Depending on the clinical examination, diabetes status and chronic periodontitis, they were divided into 3 groups:

Group I: Patients with chronic periodontitis with diabetes mellitus

Group II: Patients with chronic periodontitis without diabetes mellitus

Group III: Patients with no clinical signs of gingival inflammation.

Patients who were willing to participate in the study, Patients with history of Type 2 Diabetes mellitus , Bleeding on probing and periodontal pockets ≥ 5 mm were included.

Patients who had undergone any periodontal, surgical or non-surgical therapy or received any chemotherapeutic mouth rinse or oral irrigation during the past six months, Patients who had received antibiotics in the past 4-6 weeks or with a history of underlying systemic diseases or diagnosed with cardiovascular, cerebrovascular or kidney disease or any condition for which prophylactic treatment was done before the dental examination, Smokers, Pregnancy and lactating women and those using hormonal contraceptives were excluded in the study.

Periodontal status assessment was done by recording modified sulcular bleeding index (Modified SBI) (Mombelli et al, 1987), probing pocket depth and clinical attachment level.

Procedure for Beta Glucuronidase Estimation.

Salivary Beta glucuronidase activity was estimated by spectrophotometer using phenolphthalein glucuronic acid, acetate buffer and 2-amino-2-methyl-1-propanol buffer.

One microliter of collected saliva was transferred to small sterile plastic vial that contained 350µl of normal saline with 1% Bovine Serum Albumin.

100 microliters of 0.075M acetate buffer at pH 4.9, 50 µl of 0.03M Phenolphthalein glucuronic acid at 4.5, 50µl of saline and 50µl of sample fluid were incubated at 56^{o}C for 2 hours. The reaction was terminated by adding 350µl of 0.1M AMP(2-amino-2-methyl-1-propanol) buffer at pH 11.

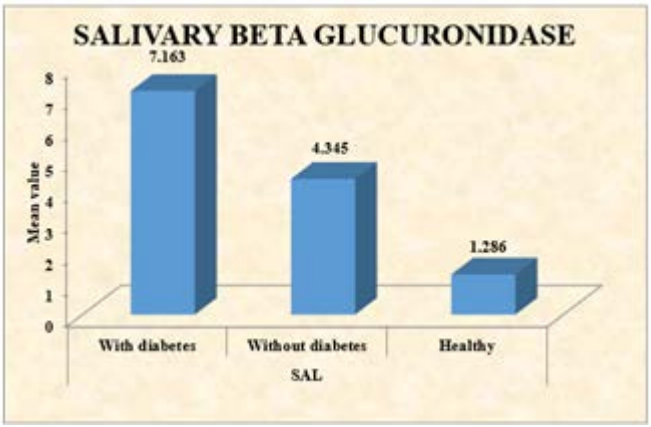
The assay was measured in the absorbance at wavelength of 550nm in spectrophotometer and compared to phenolphthalein standard curve was constructed with eight concentrations of phenolphthalein ranging from 4.0 to 0.03 µg/ml in 1:2 serial dilution (4.0, 2.0, 1.0, 0.5, 0.25, 0.12, 0.06, 0.03µg/ml) and were plotted against absorbance at 550nm in spectrophotometer.

Statistical Analysis

The statistical analysis was performed using SPSS Version 17.0 and statistical significance was defined as $p < 0.05$. The demographic analysis for distribution of age and sex was performed respectively using ANOVA and Chi square test. The comparison of clinical parameters and beta glucuronidase in all three groups were analysed using ANOVA and the multiple comparison between the groups using Tukeys test.

Results

The mean salivary beta glucuronidase was seen among the three groups. The mean beta glucuronidase was 7.163 in group I and 4.345 in group II. The mean value of group III was found to be 1.286. The mean values were decreasing accordingly and was found to be statistically very highly significant. **(Graph 1)**



Graph 1

While comparing Beta Glucuronidase Activity between groups, there was significant difference seen. In groups I and group II, the significant difference was found to be 2.818. With group III, this difference increased to 5.876. Similarly the difference between group II and group III was very highly significant difference of 3.059. **(Table 1)**

Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	p
SALIVARY BETA GLUCURONIDASE	With diabetes	Without diabetes	2.818	<0.001vhs
		Healthy	5.876	<0.001vhs
	Without diabetes	Healthy	3.059	<0.001vhs

While correlating Salivary beta glucuronidase activity with PPD, it was found that there was negative correlation with group I whereas it has positive correlation in group II and group III. Beta glucuronidase activity with CAL was found to have positive correlation with group I (r=0.347) and negative correlation in group II and III. Modified sulcular bleeding index was positively correlated(r=0.313) with Beta glucuronidase activity in group I and II whereas in group III it was negatively correlating(r=-0.17) **(Table 2).**

Group			BI	PPD	CAL
With diabetes	Salivary Beta Glucuronidase Activity	R	.050	-.197	.342
		P	.861	.481	.212
		N	15	15	15
Without diabetes	Salivary Beta Glucuronidase Activity	R	.313	.140	-.039
		P	.257	.619	.890
		N	15	15	15
Healthy	Salivary Beta Glucuronidase Activity	R	-.170	.124	-.150
		P	.544	.659	.593
		N	15	15	15

Discussion

Periodontitis is a multifactorial disease with numerous systemic or local risk factors playing a part in its clinical sequences.[11] Severe periodontitis has a prevalence of 10%–15% in the general population.[12]

An epidemiological link between diabetes and periodontitis was established in 1960.[13] Periodontitis and diabetes shares a common pathogenesis that involves an enhanced inflammatory response that can be observed at the local and systemic level. These inflammatory responses are mainly caused by the chronic effects of hyperglycemia and specifically the formation of biologically active glycated proteins and lipids that promote inflammatory responses.[14] The epidemiological evidence demonstrates that individuals with diabetes tends to have higher prevalence and more severe periodontitis than non-diabetes.[15] Uncontrolled diabetics frequently show a combination of inflammatory and degenerative changes ranging from a mild gingivitis to advanced chronic periodontitis with a widened periodontal ligament and exudation from periodontal pockets or presence of multiple periodontal abscesses.[5]

Tissue destruction as a consequence of host bacteria interaction is a well described process in the pathogenesis of periodontal diseases. During periodontal destruction, host cells mainly Polymorphonuclear leukocytes (PMN) release their granular enzymes that are capable of attacking all extracellular matrix components. Thus, extracellular presence of enzymes seems to play an important role in connective tissue damage.[16]

The present study estimated the salivary beta glucuronidase activity in chronic periodontitis with and without diabetes mellitus. The mean beta glucuronidase activity among three groups were found to be statistically very highly significant. This showed that the enzyme activity was more in Group I and less in Group III. The findings were similar to the study done by **Jaiganesh Ramamurthy et al**.[8]

Studies done by **Yoon A.J et al [17], Pushparani D.S et al [18],** found significantly higher beta glucuronidase concentration in saliva of diabetic patients when compared to non-diabetics which was in accordance with our study.

When comparing salivary beta glucuronidase activity in group I with group II and group I with group III, the latter was found to have very high significant difference (5.876, $p<0.001$)) which was similar to the studies done by **Aarthi Chowdhary et al[5] , Chandra Sekhara Prabhakar et al[20] and Richard C Oliver et al[21]**

The clinical parameters included in the study were modified sulcular bleeding Index (modified SBI), Probing pocket depth (PPD) and Clinical attachment level (CAL). In the present study, the mean values of clinical parameters in all the groups were found to have significant difference ($p<0.001$)

Beta glucuronidase activity with CAL was found to have positive correlation with group I ($r = 0.347$) which is in accordance with **Ira B Lamster et al[22]**. However Beta glucuronidase activity with CAL showed a negative correlation in group II and group III.

The limitation of the study was that it may have been underpowered due to small sample size.

Conclusion

Salivary beta glucuronidase is a potential biochemical marker of tissue destruction. The enzyme activity showed an increased level in chronic periodontitis with diabetes mellitus than non-diabetic chronic periodontitis. The increased salivary beta glucuronidase levels precedes detectable clinical changes and therefore could be a good predictor of periodontal destruction.

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