Estimation of salivary $\beta$-glucuronidase activity as a marker of periodontal disease: A case control study

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Abstract

Aim: The aim of the present study was to estimate the salivary $\beta$-glucuronidase level in healthy and diseased periodontium and to correlate the level with clinical measurement. Materials and Methods: 70 patients were included in this study with the age ranging from 30 to 65 years. Both males and females were included. They were divided into two groups: Control having healthy periodontium ($n=20$) and experimental having diseased periodontium ($n=50$). The parameters recorded were probing pocket depth, probing attachment level, gingival index, $\beta$-glucuronidase activity in the saliva, number of white blood cells, neutrophils, lymphocytes count, and platelet count. Results: It was observed that there was an increase in the level of salivary $\beta$-glucuronidase in the experimental subjects than in the control patients, and a significant positive linear relationship existed between salivary $\beta$-glucuronidase level and probing pocket depth in the experimental group. Conclusion: Level of salivary $\beta$-glucuronidase increases during inflammation in the periodontium.

Key words: $\beta$-glucuronidase, inflammation, periodontium, saliva

INTRODUCTION

Periodontal diseases are a group of inflammatory disorders that result from the host response to sub-gingival plaque microorganisms.[1] The periodontal ligament is a vascular tissue with a significant proportion of its volume made up of blood, lymph vessels and their contents.[2] Inflammation leads to the accumulation of polymorphonuclear (PMN) leukocytes, macrophages, lymphocytes, and mast cells, which are important in protecting the body against infection. These inflammatory cells contain destructive enzymes within their lysosomes, which are capable of degrading gingival tissue components, if released. Such enzymes may be released by inflammatory cells during their function or when they degenerate and die.[3]

Among the various enzyme systems that are released by inflammatory cells, $\beta$-glucuronidase is an acid hydrolase found in PMN lysosomes, which is considered to be a marker for primary granule release by these cells. Gingival crevicular fluid (GCF) has a high level of metabolic products of neutrophils. Several studies have shown that elevated GCF level of $\beta$-glucuronidase correlated with clinical periodontal parameters.[4] Layik et al. conducted a study to determine the presence and levels of $\beta$-glucuronidase activity in the gingival tissue and GCF in periodontal disease and healthy status. They found that the patient groups had higher $\beta$-glucuronidase levels in both gingival tissue and GCF than the controls. They concluded that there is a relationship between...
β-glucuronidase and periodontal disease, since increased enzyme activity in gingival tissue and GCF was observed when clinical periodontal destruction was present.[5]

The problem centered to periodontology is our inability to detect highly susceptible patients. A general approach to periodontal diagnosis that has recently been the focus of investigation in the evaluation of the pathogenic disease process is to identify the risk markers for future disease progression.[6] Efforts to develop diagnostic tests based on host factors have focused entirely on the analysis of GCF.

One of the hallmarks of current periodontal research is the search for a diagnostic test to assess periodontal disease activity through potential biomarkers which would be more predictive. GCF outflow to the oral cavity is an important contributor to the enzyme pool in whole saliva. Owing to the ease of collection of salivary samples when compared to GCF, estimating the level of the enzyme β-glucuronidase in whole saliva might be used as a marker to assess the severity of periodontal disease in the present study.

**MATERIALS AND METHODS**

**Patient selection**

The study population consisted of 70 patients of both sexes with age ranging from 30 to 65 years. Inclusion criteria included the following: Patients having pocket depth more than 5 mm and a minimum of 12 teeth excluding third molars; having no history of periodontal treatments, antiseptic therapy, or treatment with nonsteroidal anti-inflammatory drugs (NSAIDs), immunosuppressives, corticosteroids, or anti-hypertensive drug in the preceding 6 months; and patient should be a nonsmoker and free of oral lesions.

**Study design**

**Control (healthy subjects)**

The control group included 20 patients aged between 30 and 65 years with normal, clinically healthy periodontium.

**Experimental group**

The experimental group included 50 patients aged between 30 and 65 years. This group of patients was divided into two subgroups based on the mean probing depth (PD).

- **Subgroup A**
  - This subgroup consisted of 28 patients exhibiting mean PD ≤ 3.30 mm.

- **Subgroup B**
  - This subgroup consisted of 22 patients exhibiting mean PD ≥ 3.30 mm.

The same 50 patients in the experimental group were divided into two subgroups based on the mean attachment level.

- **Subgroup C**
  - This subgroup consisted of 25 patients exhibiting mean attachment level ≤ 3.30 mm.

- **Subgroup D**
  - This subgroup consists of 25 patients exhibiting mean attachment level ≥ 3.30 mm.

**Method of collection of saliva and blood**

**Saliva**

Five milliliters of unstimulated saliva was collected from the patients in new, dry, autoclaved, graduated glass test tubes that were capped with sterile tin foils and stored at −20°C till analysis.

**Blood**

Five milliliters of venous blood was collected from antecubital vein and transported to the pathology laboratory for the analysis of WBC, neutrophils, lymphocytes, and platelets.

Clinical examination was carried out using a mouth mirror, dental explorer, and a William’s periodontal probe held parallel to the long axis of the tooth. The criteria like probing depth, level of gingival attachment and the gingival index were observed Albander et al.[7]

**Biochemical analysis**

Fifty microliters of 0.9% saline control, standards, and samples were added to properly designated tubes.

For substrate standards, 0.008 mM and 0.016 mM, respectively, of 4-methyl umbelliferone was added. In each tube, 100 µl of substrate solution, i.e. methylumbelliferyl β-d-glucuronide, which contains 0.001% bovine serum albumin, was added and the contents were mixed and incubated at room temperature, i.e. 20–24°C, for 15 min. The reaction was stopped with 2 ml of 0.2 M glycine buffer at pH 11.7 containing 0.2% of sodium dodecyl sulfate. Within 60 min of mixing, fluorescence was measured with a primary wavelength of 360 nm and a secondary wavelength of 459 nm. The fluorescence of 0.008 mm of 4-methyl umbelliferone is equivalent to 1 IU of β-glucuronidase.
Glucuronidase assay

Principle
Salivary β-glucuronidase enzyme acts on the substrate methylumbelliferyl β-D-glucuronide and releases methyl umbellifereone which is a fluorescent substance and is measured in a spectrofluorometer with a primary wavelength of 360 nm and a secondary wavelength of 459 nm.

The reagents used were methylumbelliferyl β-D-glucuronide, 0.2 µl glycine buffer, pH 11.7, containing 0.2% sodium dodecyl sulfate and 4-methyl umbelliferone (standard).

Statistical analysis

- Descriptive statistics – Mean and standard deviation, minimum and maximum were calculated for all the selected variables separately for the experimental group and the control group [Table 1].
- Pearson’s correlation coefficient technique was used to find the relationship between β-glucuronidase and the selected variables [Table 2].
- Student’s “t” test was used to compare the mean β-glucuronidase level with respect to mean probing pocket depth level above 3.30 mm and below 3.30 mm between experimental and control groups [Table 3].
- Linear regression analysis was used to estimate the pocket depth level using β-glucuronidase level. [Table 4].

RESULTS

Regression analysis

Estimation of PD by β-glucuronidase level
In order to estimate the PD by β-glucuronidase level, linear regression analysis was done. The result is as follows: \( PD = 1.112 + 0.0209 \) (β-glucuronidase). This model was verified statistically and confirms the test since the standardized coefficient is 0.961 for this model: \( R^2 = (0.961)^2 = 0.924 \), i.e. 92% of the PD. This variant is significant with hat of β glucuronidase level explained by β-glucuronidase level. For example, if a person’s β-glucuronidase level is 110 IU, then PD = 1.112 + 0.0209 (110) =3.411 mm, i.e. for a person with β-glucuronidase value of 110 IU, we can expect a mean PD of 3.4 mm.

The mean gingival index was 0.43 for those having ≤3.30 mm of mean PD and 0.59 for those having >3.30 mm of mean PD, which is statistically non-significant [Table 5].

Lymphocyte count is influenced by PD
The platelet count was 2.1 for those having <3.30 mm of mean PD and 2.24 for those having >3.30 mm of mean PD, which is statistically non-significant.

Total count is influenced by attachment loss
The mean neutrophil count was 61.4 for those with ≤3.30 mm of attachment loss and 60.8 for those having >3.30 mm of attachment loss, which is statistically non-significant.

DISCUSSION

Our study population consisted of 70 patients of both sexes with age ranging from 30 to 65 years. The present study was designed to compare the salivary β-glucuronidase level between periodontally healthy and diseased subjects and also to correlate the levels of β-glucuronidase with clinical periodontal parameters such as probing pocket depth, probing attachment level, gingival index, and total leukocyte count, neutrophils, lymphocytes, and platelets in the experimental group [Table 3].

The investigation was done by collecting the salivary samples and the β-glucuronidase activity was assessed by spectrofluorometric method. The present study showed a high level of salivary β-glucuronidase activity in the experimental group than in the control group, which was similar to the results of the study conducted by Nakamura and Slots in 1983.[8] The results were also similar with those of the studies conducted in GCF by Layik et al.,[5] Lamster et al.,[9‑11] and Kaufaman.[12]

In the present study, there was a low level of salivary β-glucuronidase activity in the control patients and a significant positive relationship between salivary β-glucuronidase activity and mean PD. The
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In this study, patients were divided into subgroups based on their mean PD. Elevated salivary β-glucuronidase activity was detected in subjects with greater PD. The study also demonstrated a positive relationship between increase in salivary β-glucuronidase level and probing attachment loss in the experimental group. This was in accordance with the studies conducted in the GCF by Lamster et al.,[14] Lamster et al.,[15,16] and Layik et al.,[5] where there was a similar positive correlation between elevated β-glucuronidase activity in the GCF and probing attachment level. The patients were subdivided into two subgroups based on their mean attachment level. Elevated salivary β-glucuronidase activity was detected in subjects with greater mean attachment level.

The present study showed no significant relationship between the enzyme activity and the mean gingival index.

The present study showed no relationship between β-glucuronidase level and the total leukocyte count, which was in accordance with the study conducted by Lamster et al., 2003[16] In the present study, β-glucuronidase activity was compared with the lymphocyte count and there was a statistically significant relationship between the two, i.e., when there was increase in the β-glucuronidase activity, there was an increase in the lymphocyte count.

The present study showed no significant relationship between β-glucuronidase and the total leukocyte count, which was in accordance with the study conducted by Lamster et al.,[16]

The present study demonstrated a positive relationship between elevation in salivary β-glucuronidase level and probing attachment loss in the experimental group. This was in accordance with the studies conducted in the GCF by Layik et al.[5] Thus, from the present study, it can be inferred that salivary β-glucuronidase is a potential biochemical marker of tissue destruction. Also, there was a positive correlation between salivary β-glucuronidase activity and clinical periodontal

Table 1: Descriptive statistics with mean and standard deviation of the selected variables in the experimental group

| Variable                  | Mean    | Standard deviation | Minimum | Maximum |
|---------------------------|---------|--------------------|---------|---------|
| Age                       | 38.4000 | 8.3373             | 30.00   | 65.00   |
| Probing pocket depth      | 3.3138  | 0.2520             | 2.67    | 3.67    |
| Attachment level          | 3.3522  | 0.2822             | 2.67    | 3.90    |
| Gingival index            | 0.5012  | 0.3354             | 0.14    | 1.60    |
| Total WBC count           | 8914.000| 680.0390           | 7300.00 | 10200.00|
| Neutrophils               | 61.1600 | 4.5146             | 51.00   | 70.00   |
| Lymphocytes               | 36.6000 | 5.0467             | 30.00   | 47.00   |
| β-glucuronidase           | 2.2060  | 0.5385             | 1.10    | 3.40    |
|                          | 105.4840| 2.5204             | 101.30  | 110.00  |

WBC=White Blood Cells

Table 2: Results of Pearson’s correlation analysis between β-glucuronidase and the selected variables

| Pearson’s correlation | β-glucuronidase | P       |
|----------------------|----------------|---------|
| Probing pocket depth | 0.633          | <0.001  |
| Attachment level     | 0.713          | <0.001  |
| Gingival index       | 0.033          | 0.823   |
| Total WBC count      | 0.265          | 0.065   |
| Neutrophils          | 0.100          | 0.491   |
| Lymphocytes          | 0.639          | <0.001  |
| Platelets            | 0.141          | 0.329   |

WBC=White Blood Cells

Table 3: Comparison of β-glucuronidase levels between the experimental group and the control group

| n   | Group     | β-glucuronidase | t      | df  | P      |
|-----|-----------|-----------------|-------|-----|--------|
|     |           | Mean            | Standard deviation |
| 50  | Experimental | 105.48          | 2.52   | 177.69 | 68     | <0.001 |
| 20  | Control    | 42.1            | 2.53   |       |        |

Table 4: Mean β-glucuronidase level (IU) in patients with mean probing pocket depth and with mean attachment level above and below 3.30 mm in the experimental group

| n   | Subgroup | β-glucuronidase | Standard error mean | t   | df  | P  |
|-----|----------|-----------------|---------------------|-----|-----|----|
|     |          | Mean            | Standard deviation  |     |     |    |
| 28  | Subgroup A (MPD ≤3.30 mm) | 104.21           | 2.0244              | 0.3826 | 4.87 | 48  | <0.001 |
| 22  | Subgroup B (MPD >3.30 mm) | 107.10           | 2.1542              | 0.4593 | 6.017 | 48  | <0.001 |
| 25  | Subgroup C (MPD ≤3.30 mm) | 103.84           | 1.9107              | 0.3821 | 6.017 | 48  | <0.001 |
| 25  | Subgroup D (MPD >3.30 mm) | 107.12           | 1.9346              | 0.3869 |       |     |        |

MPD=Mean probing depth

study was also similar to the results obtained by Mc Culloch et al.,[13] in saliva.
parameters such as probing pocket depth and probing attachment loss.

**CONCLUSION**

Thus, it can be inferred from the present study that salivary β-glucuronidase is a potential biochemical marker of tissue destruction. Also, there was a positive correlation between salivary β-glucuronidase activity and clinical periodontal parameters such as probing pocket depth and probing attachment loss. The increased salivary β-glucuronidase level may be an important marker and precede detectable clinical changes and, therefore, this could be a good predictor of periodontal destruction. Significant association existed between periodontal clinical parameters and host β-glucuronidase enzyme activity in saliva, which was directly related to the inflammatory periodontal disease condition.

Based on this study, it is concluded that the activity of this enzyme increases with an increase in probing pocket depth and probing attachment level. Thus, periodontal breakdown may be attributed to increased β-glucuronidase activity in the whole saliva. Hence, the use of biochemical profile of salivary constituents offers a means of monitoring the saliva for a number of factors associated with the inflammatory response to predict periodontal disease activity. Due to non-invasive and simple nature of saliva collection, this should be further studied to determine its usefulness as a screening test for periodontitis.

A longitudinal study with even larger sample size and their interpretations. And also the influence of the microorganisms present in sub gingival areas towards the enzyme has to be evaluated earlier before considering β-glucuronidase as a definitive marker for periodontal diseases. In order to do this test one needs a basic clinic setup with a good laboratory backup and this test is cost

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**Table 5: Comparisons of gingival index, total count, neutrophil count, lymphocytes, and platelets in patients with mean probing depth and attachment level above and below 3.30 mm**

| Mean probing depth | n  | Mean | Standard deviation | Standard error mean | t    | df  | P   |
|-------------------|----|------|--------------------|---------------------|------|-----|-----|
| **Gingival index** |    |      |                    |                     |      |     |     |
| ≤3.30             | 28 | 0.4304 | 0.303               | 0.0573              | 1.718 | 48  | 0.092 |
| >3.30             | 22 | 0.5914 | 0.359               | 0.0765              |      |     |     |
| **Total count**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 28 | 9032.14 | 695.51              | 131.44              | 1.399 | 48  | 0.168 |
| >3.30             | 22 | 8763.63 | 644.05              | 137.31              |      |     |     |
| **Neutrophils**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 28 | 61.8929 | 4.6215              | 0.8734              | 1.304 | 48  | 0.198 |
| >3.30             | 22 | 60.2273 | 4.2976              | 0.9163              |      |     |     |
| **Lymphocytes**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 28 | 34.9286 | 4.017               | 0.7593              | 2.824 | 48  | 0.007 |
| >3.30             | 22 | 38.7273 | 5.496               | 1.1718              |      |     |     |
| **Platelets**     |    |      |                    |                     |      |     |     |
| ≤3.30             | 28 | 2.1964 | 0.4910              | 0.9279              | 0.140 | 48  | 0.889 |
| >3.30             | 22 | 2.2182 | 0.6052              | 0.129               |      |     |     |
| **Gingival index** |    |      |                    |                     |      |     |     |
| ≤3.30             | 25 | 0.4412 | 0.3197              | 0.0639              | 1.273 | 48  | 0.029 |
| >3.30             | 25 | 0.59612 | 0.3463             | 0.06927             |      |     |     |
| **Total count**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 25 | 9156.0000 | 582.4574          | 116.4875             | 2.66  | 48  | 0.010 |
| >3.30             | 25 | 8672.0000 | 694.9340          | 138.9868             |      |     |     |
| **Neutrophils**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 25 | 61.4800 | 4.5927              | 0.9185              | 0.497 | 48  | 0.621 |
| >3.30             | 25 | 60.8400 | 4.5063              | 0.9013              |      |     |     |
| **Lymphocytes**   |    |      |                    |                     |      |     |     |
| ≤3.30             | 25 | 34.8400 | 4.1400              | 0.8280              | 2.608 | 48  | 0.012 |
| >3.30             | 25 | 38.3600 | 5.3298              | 1.0660              |      |     |     |
| **Platelets**     |    |      |                    |                     |      |     |     |
| ≤3.30             | 25 | 2.1880 | 0.5183              | 0.1037              | 0.234 | 48  | 0.816 |
| >3.30             | 25 | 2.2240 | 0.5681              | 0.1136              |      |     |     |
effective from the patients perspective. evaluate the status of this enzyme as a definitive periodontal disease marker; but to do this test in a clinical setup, one needs to have a good laboratory backup and it is cost effective from the patients’ perspective.

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