TO ASSESS THE GCF BETA GLUCURONIDASE LEVEL IN HEALTH, LOCALIZED GINGIVITIS AND LOCALIZED ADULT PERIODONTITIS*

Dr. Surendra Man Shrestha, *MDS*
Dept. of Periodontics,
People's Dental College & Hospital
Nayabazar, Kathmandu

ABSTRACT

Gingival crevicular fluid is an inflammatory exudate that is derived from the periodontal tissue and can be collected at the orifices or from within the gingival crevice. ß-glucuronidase present in the GCF can serve as a marker for primary granule released from polymorphonuclear leukocytes. The presence of this enzyme in GCF can identify and predict probing attachment loss (PAL) in patients with untreated chronic adult periodontitis.al of twenty subjects (14 males & 6 females, mean age 35 years) with sixty sample sites were selected for the present study. Three sample sites (healthy, gingivitis and adult periodontitis) were selected from each individual in different quadrants. The result showed that the level of ß-glucuronidases increased as the severity of periodontal disease increased. Thus, the levels of ß-glucuronidase (concentration and activity) can be used as a diagnostic marker of periodontal disease.

INTRODUCTION

Gingival crevicular fluid (GCF) is an inflammatory exudate that is derived from the periodontal tissue and can be collected at the orifices or from within the gingival crevice.[8] GCF is an exudate released as a result of inflammation in the tissues adjacent to the crevicular epithelium[3]. It has been shown that clinically healthy gingiva always shows histological evidence of inflammation by accumulation of polymorphonuclear leukocytes. This is because some amount of plaque is always present in sulcular region which irritates the sulcular epithelium and the GCF accumulates in the gingival sulcus.

The potential diagnostic importance of gingival crevicular fluid was recognized more than sixty years ago. But serious investigation on the dynamics of the GCF production began in the late 1950's by Brill and co-workers. The fact that gingival crevicular fluid can be harvested from the gingival sulcus offers great potential as a source of factors associated with disease and destruction.[8,13] The

**Abstracted from a thesis presented in partial fulfillment of requirments for the award of the degree of Master of Dental Surgery (MDS) in the Faculty of Periodontics of the Rajiv Gandhi University, Bangalore, India.*

analysis of gingival crevicular fluid for disease marker has numerous advantages, because unlike serum and saliva, it is site specific, conveniently sampled and non-invasive. Tissue destruction as a consequence of host-bacterial interaction is a well described process in the pathogenesis of periodontal disease.[1] During periodontal destruction host cells (mainly polymophonuclear leukocytes) release their granular enzymes that are capable of attacking all extra-cellular matrix components. Thus, extra-cellular presence of enzymes seems to play an important role in connective tissue damage.[2] Study of these enzymes in GCF may lead to insights into the pathogenesis of periodontal disease and may provide a rationale basis for the development of noble diagnostic test. The enzymes are acid phosphatase, alkaline phosphatase, beta-glucuronidase, lysozyme, hyaluronidase, cathepsin G, collagenase, lactic dehydrogenase etc.

The assessment of periodontal disease and the effectiveness of periodontal therapy have traditionally been made using clinical and radiographic parameters. Determination of variables such as probing depth, sulcular bleeding following probing and the height of alveolar crest are the traditional basis of periodontal evaluation and periodontal treatment plan. Nevertheless, recent advances in understanding of the natural history of periodontal disease have raised questions about the significance of these diagnostic criteria. As a chronic disorder, patient with adult periodontitis will experience both active and inactive phases. Probing depth greater than particular threshold value, the presence of bleeding upon probing and radiographic evidence of loss of crestal alveolar bone have only limited value as indicator for patients, regions of the mouth, or sites at risk for future disease progression consequently. Investigators have examined other aspects of the periodontal lesion in an attempt to identify diagnostic and prognostic markers. ß -glucuronidase can serve as a marker for primary granule release from polymorphonuclear leukocytes. The presence of this enzyme in GCF was able to identify and predict probing attachment loss (PAL) in patient with untreated chronic adult periodontitis.[5,6]

AIMS AND OBJECTIVES

1. To estimate the activity and concentration of ß-glucuronidase levels in GCF in health, gingivitis and adult periodontitis sites
2. To compare the levels of ß-glucuronidase in GCF in health, gingivitis and adult periodontitis sites
3. To assess if ß-glucuronidase levels in GCF can be used as a diagnostic marker of periodontal disease

MATERIALS AND METHOD

The subjects for study were selected from the out patients visiting the Department of Periodontics, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore, India. A total of 20 subjects (14 males and 6 females, mean age 35 years, range 30 to 42 years) with a total of 60 sample sites were selected. Three sample sites were selected from each subject (healthy, gingivitis and adult periodontitis) in different quadrants. Total 60 sites were divided into 3 equal groups (20 sites of healthy, 20 sites of gingivitis and 20 sites of adult periodontitis).

CRITERIA FOR PATIENT SELECTION:

1. Gingival scores for the selected sites were estimated
   using Gingival Index by Loe and Silness (1967)[9]
2. Pocket depths were measured using William's graduated periodontal probe. Patients with periodontal pocket depth ranging between 5-8 mm were selected for the study in experimental groups

3. Control groups were normal and healthy individuals

CRITERIA FOR GROUP DIVISION:

Group I (Clinically healthy gingiva): The marginal gingiva is pink, firm, visually free of inflammation and do not bleed on probing and marginal gingiva is coronal to CEJ. Sulcus depth is 3 mm or less.

Group II (Gingivitis): This group included only plaque associated gingivitis. The marginal gingiva is erythematous and edematous with bleeding on probing. The sites exhibited no clinical attachment loss. Sulcus depths are less than or equal to 3 mm and gingival bleeding score is equal or more than 2.0 and are determined by the gingival index (Loe & Silness, 1967).[26] The marginal gingiva is coronal to CEJ.

Group III (Adult periodontitis): Clinical loss of attachment is present (base of the pocket is apical to CEJ). Pocket depth should be 5 to 8 mm with bleeding on probing. A gingival bleeding score greater than or equal to 2.0 is required and in determined by the gingival index (Loe & Silness, 1967).

THE BIOCHEMICAL LABORATORY PROCEDURE: [4]

The ß-glucuronidase concentration (u/µl) and activity (u/30 secs) in the diluted GCF sample was determined in spectrophotometer. One micro liter of collected GCF was transferred to small sterile plastic vial that contained 350 micro liter of normal saline with 1% bovine serum albumin. 100 micro liter of 0.075 M acetate buffer at pH 4.9, 50 micro liter of 0.03 M phenolphthalein glucuronic acid at pH 4.5, 5.0 micro liter of saline and 50 microliter of sample fluid were incubated at 56°c for 2 hours. The reaction was terminated by adding 350 micro liter of 0.1 M AMP (2-amino-2-methyl-1-propanol) buffer at pH 11.

The assay was measured in the absorbance at wave length of 550 nm in spectrophotometer and compared to phenolphthalein standard curve to obtain concentration result. Phenolphthalein standard curve was constructed with eight concentration of phenolphthalein ranging from 4.0 to 0.03 microgram/ml in 1:2 serial dilutions (4.0, 2.0, 1.0, 0.5, 0.25, 0.12, 0.06, 0.03 microgram/ml) and were plotted against absorbance at 550 nm in spectrophotometer.

RESULT

Table – I Descriptive Statistics

Groups	Age (Mean ± SD)	Pocket depth /Sulucs depth (Mean ± SD)	ß-Glueuronidase concentration (Mean ± SD)	ß-Glueuronidase activity (Mean ± SD)
Healthy	35 ± 3.3	3 ± 0.31	0.432 ± 0.216	0.042 ± 0.032
Gingivitis	35 ± 3.3	3 ± 0.00	1.283 ± 0.86	0.222 ± 0.050
Adult periodontitis	35 ± 3.3	6.2 ±1.11	2.090 ± 0.413	0.602 ± 0.173

Table – II ANOVA Test

	F- Ratio	P	Remarks
ß-Glueuronidase concentration	136.534	< 0.001	VHS
ß-Glueuronidase activity	148.454	< 0.001	VHS

(VHS-very highly significant)

Table – III Comparison of mean ß-Glucuronidase concentration level using unpaired student 't' test[10]

Group comparison	T	P	Remarks
Healthy (Group I) Vs Gingivitis (Group II)	10.37	< 0.001	VHS
Healthy (Group I) Vs Periodontitis (Group III)	15.94	< 0.001	VHS
Gingivits (Group II) Vs Periodontitis (Group III)	7.14	< 0.001	VHS

Group – IV Comparison of mean ß-Glucuronidase activity level using unpaired student 't' test

Group comparison	T	P	Remarks
Healthy (Group I) VS Gingivitis (Group II)	4.22	< 0.001	VHS
Healthy (Group I) VS Periodontitis (Group III)	11.28	< 0.001	VHS
Gingivits (Group II) VS Periodontitis (Group III)	9.60	< 0.001	VHS

Chart 1

COMPARISON OF MEAN - GLUCURONIDASE CONCENTRATION LEVEL OF THREE GROUPS

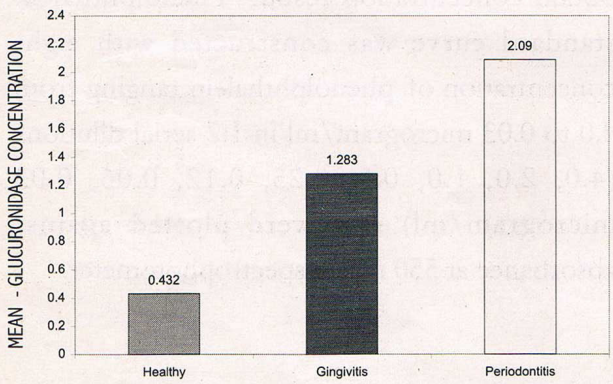
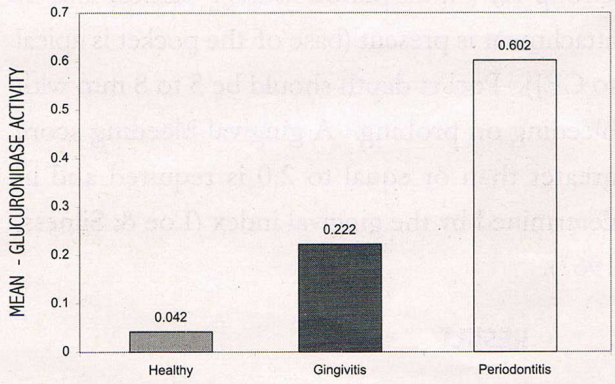


Chart 1

COMPARISON OF MEAN - GLUCURONIDASE ACTIVITY LEVEL OF THREE GROUPS



According to Table I, the mean ß-Glucuronidase concentration and activity in healthy group (Group I) are 0.432 and 0.042 with a standard deviation of ± 0.216 and ± 0.032 respectively. In the gingivitis group (Group II), the mean ß-Glucuronidase concentration and activity are 1.283 and 0.222 with a standard deviation of ± 0.86 and ± 0.050 respectively. In the periodontitis group (Group III) the mean ß-Glucuronidase concentration and activity are 2.090 and 0.602 with a standard deviation ± 0.413 and ± 0.173 respectively.

Table II shows the ANOVA test, which is the test of significance. It was done for ß-Glucuronidase concentration and ß-Glucuronidase activity levels respectively. F-ratio of ß-Glucuronidase concentration and activity are 136.534 and 148.454 respectively. The ß-Glucuronidase concentration and activity levels were found to be very highly significant.

Table III shows unpaired student 't' test for comparison of mean ß-Glueuronidase concentrations between group I and group II,

which showed 't' value of 10.37. Comparison between group I and group III showed 't' value of 15.94. Comparison between group II group III showed 't' value of 7.14. All these comparisons were very highly significant ($P<0.001$). Table IV shows the unpaired student 't' test for comparison of mean ß-Glucuronidase activity. Comparison between group I and group II showed 't' value of 4.22. Comparison between group I and group III showed 't' value of 11.28. Comparison between group II and group III showed 't' value of 9.60. All these comparisons were very highly significant ($P<0.001$).

Chart I shows the comparison of mean values of ß-Glucuronidase concentration levels in health, gingivitis and adult periodontitis. The values in periodontitis is highest followed by gingivitis and healthy respectively.

Chart II shows the mean values of ß-Glucuronidase activity levels in health, gingivitis and periodontitis. The highest value was observed in periodontitis followed by gingivitis and in health respectively. Thus from the above statistical analysis, a conclusion can be drawn that the levels of ß-Glucuronidases increases as the severity of disease increases. Thus, the levels of ß-Glucuronidase (concentration and activity) can be used as a diagnostic marker of periodontal disease.

DISCUSSION

It is universally accepted beyond question that periodontal disease is a response of the tissues to invading microbes present in the dental plaque. The first line of defense against any invasion is by the PMNLs which are chemotactically attracted to the affected sites. The result of interaction between the PMNLs and bacteria release various enzymes from both microbes and host cells. One of these enzymes which is commonly seen in area of destruction is ß-Glucuronidase. It is host derived and polysaccharide hydrolyzing enzyme. It is present in the azurophilic granules of PMNLs and is released during host bacterial interaction. Concentration and activity of ß-Glucuronidase in GCF provides an indication of neutrophil influx into the crevicular environment. It is one of the enzymes involved in the destruction of non-collagenous component of the extra-cellular matrix. It is significantly elevated in individuals with gingivitis and periodontitis. It's concentration and activity correlates with periodontal disease severity.[11]

Periodontal disease is no longer considered a continuously progressive lesion, but is one which occurs in an episodic pattern, with periods of exacerbation and remission, in which there is a complex interaction between the periodontal pathogens and defense mechanism of the host. The exacerbation may take the form of random bursts, multiple bursts or other forms. The alternating chronic and episodic nature of periodontal disease, the wide range of disease severity affecting different teeth within the same subject and the prolonged requirement for repeated treatments makes it necessary to augment traditional diagnostic aids like clinical examination, probing depth, attachment loss, radiographs and by biochemical assays utilizing biochemical diagnostic markers.

In the present investigation, the concentration and activity of ß-Glucuronidase in GCF was estimated. As microcapillary pipettes were used the problems of using filter paper (Nakashima, Demeurisse, Cimasoni 1994)[12] were avoided, which include imperative use of detergents, agitation, centrifugation, and the undesirable chromatographic separation of GCF constituents. Further, use of the Periotron creates problem of having to convert Periotron units into micro liters, sample loss on the surface of the Periotron plates. The regular calibration is needed for each sample material being used because Periotron readings

vary with room temperature, relative humidity and sample composition. Besides this, the Periotron gives an initial reading and the maximum reading appears after one minute or even 5 ½ minutes and not after a fixed interval of time .

In the present study the ß-Glucuronidase levels in GCF expressed as concentration (u/µl) and activity (u/30 sec) were estimated in 60 sites. ß-Glueuronidase concentration refers to the enzyme collected per micro liter of GCF fluid (u/µl) and ß-Glucuronidase enzyme activity refers to the amount of enzyme collected in 30 seconds.

In each group, the gingival index, pocket /sulcus depth, and ß-Glucuronidase levels were calculated, gingival score was a constant (GI = 2,) except for the control group. The sulcus depth was < 3 mm except periodontitis sites. The pocket depths ranged from 5 to 8 mm in periodontitis sites. The mean and standard deviation of the age, ß-Glueuronidase concentration and ß-Glucuronidase activity were calculated in all three groups.

ANOVA test was done to find out if there was any significance of ß-Glucuronidase concentration and activity in gingivitis and periodontitis sites comparing with control group. A very highly significant difference of ß-Glucuronidase concentration and ß-Glucuronidase activity among three groups ($P < 0.001$ VHS) was observed in the study.

Unpaired student 't' test was done to compare the concentration and activity of ß-Glucuronidase among healthy vs gingivitis, healthy vs periodontitis and gingivitis vs periodontitis respectively. The results were very highly significant ($P < 0.001$ VHS).

Chart I showed the comparison of mean values of ß-Glucuronidase concentration in healthy, gingivitis and periodontitis sites. Here results were highest in periodontitis, followed by gingivitis and control group respectively.

The findings of the present study confirm the relationship between ß-Glucuronidase level and periodontal disease, since increased enzyme activity in GCF was observed when clinical periodontal destruction was present. However, the diseased groups presented higher enzyme activity than the healthy sites. Similar results were observed with ß-Glucuronidase activity levels in GCF samples as well. Therefore, these findings support the role for increasing ß-Glucuronidase levels in the pathogenesis of periodontal disease.

The highest ß-Glucuronidase concentration and activity in GCF was found in periodontitis sites. This increase in periodontitis forms can be attributed to the hyperactive state and pronounced response of PMN as a consequence of severity of microbial virulence factors and also to the lytic effect of more pathogenic sub-gingival bacteria and host cells leading to an intense host enzyme release. Lamster et al[23] have suggested that elevated

ß-Glueuronidase level could be invaluable in the identification of patients at risk for active periodontal disease.

Findings in this study showed that ß-Glueuronidase activity is a time-based measurement and ß-Glucuronidase concentration is a volume-based measurement. It was precise enough to demonstrate the statistical differences among all the groups. Therefore, results of this study further strengthened the fact that as periodontal destruction increases, concentration and activity of ß-Glucuronidase in gingival crevicular fluid also increases. Hence from this study, it may be deduced that ß-Glucuronidase is a potential biochemical marker of periodontal disease activity.

SUMMARY AND CONCLUSION

The present study was conducted in the epartment of Periodontics, A. B. Shetty Memorial Institute of Dental Sciences, Mangalore to estimate the concentration and activity of ß-Glucuronidase in GCF in health, gingivitis and adult periodontitis sites.

The study sample consisted of 60 sites which were divided into three groups; 20 sites in healthy group, 20 sites in gingivitis group and 20 sites in periodontitis group. The criteria for patient selection were Gingival index (Loe & Silness) = 2, except in healthy group and pocket depth 5 to 8 mm for periodontitis group. Micropipettes were used for collection of GCF and the spectrophotometer was used to estimate the concentration of ß-Glueuronidase.

From the results obtained, following conclusions were drawn:

1. Analysis of ß-Glucuronidase in GCF in health, gingivitis and adult periodontitis groups reveal that the levels were highest in adult periodontitis followed by gingivitis and healthy sites respectively
2. ß-Glucuronidase concentration and activity in GCF can be used as biochemical marker for periodontal disease activity

However, since periodontal disease involves other etiological factors like microorganisms and host defense mechanism, so ß-Glucuronidase alone cannot be considered as a clinical biochemical disease marker. Due to the presence of micro-organisms, a variety of enzymes are released that contribute to the destructive process. ß-Glucuronidase is a glycoprotein produced by many cells within the area of the periodontium and gingival crevice. The main sources of the enzymes are ploymorphonuclear leukocytes and bacteria within the supra- and sub-gingival plaque. It is one of the enzymes involved in the destruction of non-collagenous components of the extra-cellular matrix. Thus it may be used as a biochemical and diagnostic marker for periodontal disease activity.

Further studies on the predictability and sensitivity of ß-Glucuronidase as a biochemical marker for periodontal disease progression are required.

REFERENCES

1. Cimasoni G.: Crevicular fluid updated vol. 12 Basel Karger II Edn 1983; 24-28, 74-102.
2. Daneshmand H and Bryan wade A: Correlation between gingival fluid measurements and microscopic and macroscopic characteristics of gingival tissue. *J. Periodont Res* 1976, 11:35-46.
3. Fine DH and Mandel ID: Indicators of periodontal disease activity: An evaluation *J. Clin Periodontol* 1986, 13:533-546.
4. Lamster I.B.: Evaluation and modification of spectrophotometric procedures for analysis of lactate dehydrogenase, Beta-glucuronidase and Arylsulphatase in Human gingival crevicular fluid collected with filter-paper strips. Archs oral Biology 1985 vol 30, No 3, 235-242.
5. Lamster I.B., Oshrain H.L., Harper D.S., Celenti R.S., Hoviliarus C.A. and Gordon J.M.: Enzyme activity in crevicular fluid for detection and prediction of clinical attachment loss in patients with chronic adult periodontitis. *J Periodontol* Aug 1988; 516-523.
6. Lamster I.B. Oshrain R.L., celenti RS, Fine JB. Garbic JT: Indicators of the acute inflammatory and humoral responses in gingival crevicular fluid: Relationship to active periodontal disease *J. Periodont Res* 1991;26: 261-263.
7. Lamster IB, Holmes LG, Gross KBW, Oshrain RL, Cohen DW, Rose LF, Peters LM and pope MR: the relationship of ß-glucuronidase activity in crevicular fluid to clinical parameters of periodontal disease. Findings from a multicenter study *J. Clin Periodontol* 1994; 21: 118-127.
8. Lamster IB: Host responses in GCF potential applications in periodontitis clinical trial. *J. Periodontol* 1992: 63:177-123.
9. LOE H.: The gingival index, the plaque index and the retention index systems. *J. Periodontol* 1967, 38, 610-616.
10. Mahajan B.K. Methods in Biostatistics for medical students and Research Workers. 1997, 6th Edition, Jaypee Brothers medical Publishers (P) Ltd.
11. Meral Layik, Nermin Yamalic, Feriha Caglayan, Kamer Kiline, Ilker Etican, and Eratalay : Analysis of Human gingival tissue and gingival crevicular fluid - Glucuronidase activity in specific periodontal disease. *J. Periodontol* 2000, 71, 618-624.
12. Nakashima K, Giannopoulou C, Andersen E, Roehrich N, Brochut P, Dubrez B, Cimasoni G: A longitudinal study of various crevicular fluid components as markers of periodontal disease activity *J. Clin Periodontal* 1996; 23: 832-838.
13. Page R.C: Host response tests for diagnosis of periodontal disease. *J. Periodontal* 1992, 63:356-366.