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EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON SALIVARY BETA GLUCURONIDASE AND CHRONIC PERIODONTITIS WITH AND WITHOUT SMOKELESS TOBACCO LINKAGE: A LONGITUDINAL STUDY"

Dental Science

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ABSTRACT

INTRODUCTION: Beta glucuronidase (βG) is one of the enzymes involved in the destruction of non- collagenous components of extra cellular matrix that contributes significantly to tissue destruction and the pathogenesis of periodontal disease. **OBJECTIVE :** Aim of the study was to estimate the effect of non-surgical periodontal therapy (NSPT) on salivary βG in chronic periodontitis (CP) with and without smokeless tobacco (ST) and to evaluate the relationship between beta glucuronidase activity and periodontal clinical parameters. **METHODOLOGY:** The study consisted of 100 subjects (25 each) - healthy, gingivitis, CP with and without ST. Clinical parameters and salivary βG were measured at baseline and 4 weeks after non- surgical periodontal therapy using spectrophotometer at 550 nm. Statistical analysis was carried out using paired t test, chi-square , analysis of variance (ANOVA). **RESULTS:** The mean βG values in saliva were found to be significantly higher in periodontitis with smokeless tobacco subjects after 1 month NSPT, compared at baseline. ($p<0.001$) CONCLUSION: Successful NSPT lead to progress in inflammatory condition, regulating βG levels & effect of ST on periodontium. Thus, can be used as potent biomarker for periodontal disease activity.

KEYWORDS

Beta glucuronidase; Periodontitis; Smokeless tobacco

INTRODUCTION:

Periodontal diseases are a group of inflammatory disorders that result from the host response to sub-gingival plaque microorganisms. 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.[1]

Among the various enzyme that are released by inflammatory cells, beta glucuronidase is considered to be a marker for primary granule release by neutrophils. Beta glucuronidase is a PMN derived lysosomal acid hydrolase, which is stored in primary azurophil granules and it contributes to non-collagenous matrix degradation in periodontal diseases.[2]

Beta- glucuronidase (βG) in gingival crevicular fluid, a marker of neutrophil influx into the crevicular environment, has previously been shown to be correlated with periodontal clinical parameters and periodontal attachment loss. Analysis of βG in saliva may be measure of crevicular neutrophil influx for whole mouth. Thus, use of analysing saliva for periodontal diagnosis has been the subject of interest. Saliva is a fluid that is readily available and contains locally and systemically derived markers of periodontal disease and hence may offer the basis for a patient-specific diagnostic test for periodontitis.3,4 The association between smokeless tobacco and chronic periodontitis has received evidential support over the years. The susceptibility of individuals with smokeless tobacco habit to develop chronic periodontitis has been reported to be higher than that in healthy subjects.[5]

Mechanical debridement has been the corner-stone for professional plaque control and prevention of periodontal disease for centuries. Successful periodontal treatment has shown to reduce the inflammatory burden and decrease the salivary levels of beta glucuronidase Thus, the present interventional trial was carried out to discover if the improvement in periodontal status after scaling and root planing could result in any alteration of the salivary beta glucuronidase activity in healthy, gingivitis, chronic periodontitis with and without smokeless tobacco subjects. By this, it not only preserves periodontal tissues, but also helps to limit the oral source of inflammation contributing to overall systemic well–being, as well as it enhances the maintenance of periodontal health.

MATERIALS AND METHODS

STUDY POPULATION

The power of this study was 80%, at 95% confidence interval and margin of alpha error being 5%, beta error being 20% a sample size of participants was derived. Subjects, males and females in the age group 20 to 65 years were randomly selected from the outpatient section of Department of Periodontics, P.M.N.M. Dental College, Bagalkot, Karnataka, India. The study was approved by the Institutional Ethical Committee. All potential participants were clearly explained regarding the need and design of the study.

A written duly signed informed consent was obtained from the participants and were divided into 4 groups of 25 each as, Group I: Healthy subjects who had no systemic diseases and showed absence of clinical and radiographic signs of periodontitis and with atleast 20 teeth present. Group II: Gingivitis subjects with plaque present at gingival margin, change in color, contour, bleeding on probing (BOP) on provocation.6 Group III: Chronic periodontitis subjects with BOP, CAL ≥3 mm at > 30% sites in the mouth ,atleast 20 teeth present.6 Group IV: Smokeless tobacco with chronic periodontitis subjects who are current tobacco users, who reported snuff or chewing tobacco use at least 20 times and who reported using snuff or chewing tobacco at the time of interview.7 Group II, III, IV subjects were recalled after 1 month of non surgical periodontal therapy.[8]

Patients with a history of any antibiotic/anti-inflammatory and corticosteroid therapy for 6 months prior to study, any known systemic disease or conditions, history of cigarette smoking, alcohol consumption, periodontal therapy 6 months prior to study and pregnant/lactating women were excluded from the study.9 A short-

structured case history was recorded for each subject to obtain information on age, sex, occupation, cigarette smoking and drug history. It included details of smokeless tobacco consumption (duration, type & frequency), dietary supplements such as vitamins, drug intake, alcohol, medical history etc.[10]

DETERMINATION OF CLINICAL PARAMETERS

Full mouth periodontal examination was performed by a single examiner after proper grouping of the subjects. The periodontal parameters like gingival index (GI) (Loe and Silness 1963)11, Plaque index (PI)12 , bleeding on probing (BOP)/ bleeding index (Muhlemann H R and Son S1971)13, pocket probing depth (PPD) (Russell AL 1956)14, clinical attachment level (CAL) were assessed using a Williams periodontal probe by a single examiner.

SALIVA SAMPLE COLLECTION

About 5ml of unstimulated saliva was collected from each subject in new, dry, autoclaved, glass test tubes that were capped with sterile tin foils. To minimize circadian influences, sample collection was done between 09:00 a.m to 11:00 a.m at least 1 hour after eating or washing of mouth. During collection subjects were comfortably seated and were instructed to rinse their mouth thoroughly with water, with head tilted slightly down. Saliva was allowed to pool in the floor of the mouth for 5minutes by leaning forward. Subjects were then told to spit into glass test tube every 60 seconds for 10 minutes or when the subject experienced an urge to swallow the fluid accumulated in the floor of the mouth and stored at -20 degree celsius till analysis.9

PERIODONTAL TREATMENT

All patients were motivated and instructed in daily plaque control. Nonsurgical periodontal therapy was performed on patients with gingivitis, chronic periodontitis with and without smokeless tobacco. Phase I therapy included pre procedural oral rinse, full mouth scaling and root planning along maintenance and monitoring of oral hygiene. Clinical data and samples were obtained at baseline and at 1 month after non-surgical periodontal therapy.[8]

ESTIMATION OF SALIVARY BETA GLUCURONIDASE ACTIVITY

Fifty micro liters of control, standards and samples was added to properly designated tubes. Unionized distilled water was used as control. Phenolphthalein glucuronic acid was used as standard and patient's saliva was used as sample. With the help of micropipette, 50µl of saliva was taken in a cuvette. To this 50µl of 25mM of phosphate buffer at pH 4.9 and phenolphthalein glucuronic acid was added. The tube was incubated at 65°C for one hour. The acidic assay permits elimination of the bacterial enzyme activity. The reaction was terminated by the addition of 100 µl of 200 mM glycine buffer (pH11). The intensity of red color was measured at 550 nm in spectrophotometer. The β glucuronidase activity was measured in units (U).[9]

STATISTICAL ANALYSIS

The data collected was analysed using computer software IBM SPSS statistics 20.0. Descriptive and inferential statistics was carried out. Chi square test was used to know the gender distribution; Paired t test was used to find the significance of study parameters on continuous scale between two groups (Inter and Intra group analysis) on metric parameters. ANOVA was used to find the significance of study parameters between the groups (Inter group analysis) at baseline, and non parametric test Kruskal Wallis test was used as part of the data was not following the normal distribution between the groups (Inter group analysis) at baseline and 1 month after non surgical periodontal therapy. Data were expressed as mean and standard deviation.

RESULTS

A total of 100 subjects, age ranging from 25 to 65 years among which 17 females and 8 males in healthy with mean age of 30.12±7.59 years, 15 females and 10 males in gingivitis with mean age of 33.48±8.91 years, 12 females and 13 males in chronic periodontitis with mean age of 45.04±11.23, 9 females and 16 males in chronic periodontitis with smokeless tobacco with mean age of 43.12±11.60 years were enrolled in this longitudinal biochemical study. Age and sex matching was not possible, as subjects were recruited for the study as and when they came to the college outpatient department for dental examination.

The mean clinical parameters showed at different time intervals i.e. at baseline and 1 month after non surgical periodontal therapy in gingivitis, were found to be highly significant where as pocket probing depth showed non-significant and clinical attachment loss [Table 1].

The mean clinical parameters i.e. gingival index, plaque index, bleeding index, probing pocket depth, clinical attachment level & βG values in terms of {Mean (SD)} at different time intervals i.e at baseline and 1 month after non surgical periodontal therapy in chronic periodontitis with and without smokeless tobacco were found to be highly significant ($p<0.001$) [Table 1].

Table 1: Comparison of the clinical parameters & beta glucuronidase levels at different time intervals in Gingivitis, Chronic periodontitis with and without Smokeless tobacco group using paired t test

Gingivitis group						
Variables	Time interval	N	Mean	Std. Deviation	t value	P value
Gingival Index	Baseline	25	1.0600	0.55951	4.081	<0.001**
	1 month	25	0.815	0.4416		
Plaque Index	Baseline	25	1.255	0.5296	4.268	<0.001**
	1 month	25	0.980	0.3955		
Bleeding Index	Baseline	25	1.405	0.5708	5.128	<0.001**
	1 month	25	1.055	0.4430		
Probing Pocket Depth	Baseline	25	2.35	0.489	1.285	0.214(NS)
	1 month	25	2.15	0.366		
Clinical Attachment	Baseline	25	0	0.0	NA	NA
	1 month	25	0	0.0		
Beta Glutathione	Baseline	25	2.1680	0.57757	4.874	<0.001**
	1 month	25	1.7580	0.45809		
Chronic periodontitis group						
Gingival Index	Baseline	25	2.1950	0.20894	4.483	<0.001**
	1 month	25	1.870	0.3975		
Plaque Index	Baseline	25	2.420	0.2118	5.800	<0.001**
	1 month	25	2.225	0.1618		
Bleeding Index	Baseline	25	2.580	0.2167	5.217	<0.001**
	1 month	25	2.365	0.1872		
Probing Pocket Depth	Baseline	25	6.70	0.907	6.540	<0.001**
	1 month	25	4.98	0.709		
Clinical Attachment loss	Baseline	25	7.58	0.858	9.516	<0.001**
	1 month	25	5.09	0.779		
Beta Glucuronidase	Baseline	25	3.0980	1.06477	6.015	<0.001**
	1 month	25	2.2410	0.72927		
Chronic periodontitis with Smokeless tobacco						
Gingival Index	Baseline	25	2.2600	0.38443	6.136	<0.001**
	1 month	25	1.900	0.3960		
Plaque Index	Baseline	25	2.265	0.3281	2.595	0.018*
	1 month	25	2.040	0.3719		
Bleeding Index	Baseline	25	2.700	0.3836	6.422	<0.001**
	1 month	25	2.375	0.3462		
Probing Pocket Depth	Baseline	25	7.48	1.147	6.802	<0.001**
Clinical Attachment loss	1 month	25	6.38	0.967		
	Baseline	25	8.02	1.082	9.338	<0.001**
	1 month	25	6.47	0.970		
Beta Glucuronidase	Baseline	25	3.2780	0.73174	8.310	<0.001**
	1 month	25	2.5175	0.49212		

b($p< 0.05$ – Significant*, $p < 0.001$ - Highly significant**)

The mean clinical parameters i.e. gingival index, plaque index, bleeding index, probing pocket depth, clinical attachment level & an inflammatory marker βG in unstimulated saliva in terms of {Mean (SD)} were found to be highly significant ($p<0.001$) with positive correlation between clinical parameters and βG, as shown in at baseline among all the 3 groups, as shown in [Table 2].

Table 2: Comparison of the clinical parameters & beta glucuronidase levels at baseline among all the 4 groups using ANOVA test

Variables	Healthy	Gingivitis	CP	CP with Smokeless tobacco	F value	P value
GI	0.30±0.21	1.06±0.5	2.1±0.2	2.2±0.3	128.69	<0.001**
PI	0.37±0.22	1.25±0.32	2.4±0.2	2.2±0.32	151.99	<0.001**
BOP	0.28±0.24	1.40±0.57	2.5±0.21	2.7±0.38	177.77	<0.001**
PPD (mm)	2.0±0.0	2.35±0.48	6.70±0.9	7.48±1.14	275.35	<0.001**
CAL (mm)	0.0±0.0	0.0±0.0	7.58±0.85	8.02±1.08	852.32	<0.001**
Beta Glucuronidase	0.06±0.01	2.16±0.57	3.09±1.06	3.27±0.73	86.61	<0.001**

b* mm – Millimeter (p < 0.001 - Highly significant**)

When comparision was made at baseline and 1 month after non surgical periodontal therapy, there was a positive correlation between study groups and clinical parameters. Where as GI, plaque index and BOP showed statistically non significant values and PPD, CAL and βG showed highly significant values (p<0.001), as shown in [Table 3]

Table 3: Comparison of the mean difference (Baseline – 1 month) of clinical parameters & beta glucuronidase levels among all the 3 groups using Kruskal Wallis test

Variables	Gingivitis	CP	CP with ST	Chi square value	P value
GI	0.24±0.26	0.32±0.32	0.36±0.26	3.040	0.219 (NS)
PI	0.27±0.28	0.19±0.15	0.22±0.38	0.272	0.873 (NS)
BOP	0.35±0.30	0.21±0.18	0.32±0.22	1.296	0.523 (NS)
PPD (mm)	0.20±0.69	1.72±1.17	1.10±0.72	19.63	<0.001**
CAL (mm)	0.0±0.0	2.49±1.17	1.55±0.74	44.23	<0.001**
Beta Glucuronidase	0.41±0.37	0.85±0.63	0.76±0.4	9.89	0.007*

mm – Millimeter(NS – Non Significant, p< 0.05 – Significant*, p < 0.001- Highly significant**)

DISSCUSSION

Pathogenesis of periodontal diseases is well described by host bacterial interaction. Host cells during periodontal tissue destruction, mainly polymorphonuclear leukocytes (PMN) release their azurophilic granular enzymes that are proficient in attacking all extracellular matrix components. β Glucuronidase together with hyaluronidase is involved in the catabolism of proteoglycans. The endoglycosidase hyaluronidase cleaves hexosaminidic linkages, producing tetrasaccharides with the structure of (GlcUA-β1, 3 - GlcNAcβ1,4). This tetrasaccharide is further degraded by β Glucuronidase and β-N-acetyl hexosaminidase. β Glucuronidase is an exoglycosidase that removes GlcUA from non reducing ends of tetrasaccharides or larger polysaccharides. Therefore, it contributes to non-collagenous matrix degradation in periodontal diseases.15,16

Among all markers for periodontal disease, host derived enzymes in saliva appear to be best indicators as found by Kaufman and Lamster17 and was also well supported by kinney et al.18 Our study consisted of 100 subjects age ranging from 20-65 of both sex and they are divided into four groups. The mean BG activity of Group IV was significantly higher than Group III, II and I. Also there was a high level of salivary β glucuronidase in periodontitis patients compared to gingivitis and healthy controls. Even though, the salivary BG activity was present in healthy controls and gingivitis group; the values were significantly lesser than periodontitis group. This shows that beta glucuronidase activity is related to attachment loss. The results were also similar with the study conducted in GCF by Layik et al.15, Lamster IB et al.19, Nakamura M et al.20 and SK Balaji et al.21 There was a low level of salivary BG activity in control patients that was similar to the study conducted in GCF by Lamster IB et al.22

The Periodontal status i.e. clinical parameters- G.I, PI, BOP at baseline and 1 month after non surgical periodontal therapy were similar for Group II, Group III and Group IV (no statistical significant differences at $p < 0.05$). But the Beta Glucuronidase activity was significantly higher in Group IV when compared to Group III, Group II. So this increase in βG activity in Group IV could be due to smokeless tobacco which were in accordance with the study done by Ali SA et al.23 and Pabst MJ et al24 that is nicotine affected oxygen dependent killing mechanisms, inhibiton of aerobic and anaerobic antimicrobial functions of neutrophils and monocytes by nicotine alter the microbial ecology of oral cavity, and compromises oral health. Decrease in βG activity in Group IV after 1month of non surgical periodontal therapy could be due to reduction in microbial load on periodontium, thus improving periodontal health which was in concordance with Proye M et al.25

Limitations of the present study been the smaller sample size. Gender distribution was not possible as patients were randomly selected from the outpatient department. And various other differences such as race and population variations don't allow generalizing these findings on to the entire population. Therefore, further studies with larger sample size, equal gender distribution and on a multi-center level including population of various races are required to consolidate the findings of the present study.

CONCLUSION:

The current study found that salivary β-glucuronidase enzyme activity significantly increased among patients with periodontal disease with and without smokeless tobacco in comparison to those with no/mild gingival inflammation. While smokeless tobacco significantly influenced enzyme activity, these changes were within acceptable limits, and also successful non surgical periodontal therapy improved the periodontal condition by reducing β-glucuronidase activity and thus, acts as diagnostic biomarker of periodontitis.

Conflict of interest: None as such.

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REFERENCES:

11. Prabhahar CS, Mohamed Niazi KT , Prakash R, Yuvaraj A, Goud S and Ravishekar P. Estimation of salivary β-glucuronidase activity as a marker of periodontal disease: A case control study. J Int Soc Prev Community Dent. 2014;4(3):S193–S198. doi: 10.4103/2231-0762.149039
2. Layik M, Yamalik N, Caglayan. Analysis of human gingival tissue and gingival crevicular fluid β glucuronidase activity in specific periodontal diseases. J Periodontol. 2000;71:618-24. DOI: 10.1902/jop.2000.71.4.618
3. Lamster IB, Kaufman E, Grbic JT, Winston LJ and Singer RE. β- Glucuronidase activity in saliva: Relationship to clinical periodontal parameters. J Periodontal 2003;353-359. DOI: 10.1902/jop.2003.74.3.353
4. Kaufman E, Lamster IB. Analysis of saliva for Periodontal diagnosis. J Clin Periodontol. 2000; 27:453-65. DOI: 10.1034/j.1600-051x.2000.027007453.x
5. Katuri KK, Alluri JK, Chintagunta C, Tadiboina N, Borugadda R, Loya M et al. Assessment of periodontal health status in smokers and smokeless tobacco users: A cross sectional study. J Clin Diagn Res. 2016;10(10):ZC143-ZC146 doi: 10.7860/JCDR/2016/22160.8700
6. American Academy of Periodontology Task Force Report on the Update to the 1999 classification of Periodontal Diseases and Conditions. J Periodontol 2015; 86(7):835-838. DOI: 10.1902/jop.2015.157001
7. Use of Smokeless tobacco among adults- United States, 1991, CDC April 1993;42:263-66. https://www.cdc.gov/mmwr/preview/mmwrhtml/00020232.htm
8. Dagar M, Deepa DK, Molly M, Sharma A and Khattak BP. Effect of non-surgical periodontal therapy on salivary myeloperoxidase levels: a biochemical study. J Indian Soc Periodontol.2015;19(5):531-536. doi: 10.4103/0972-124X.162199
9. Ramamurthy J, Jayakumar Nd, Varghese S. Comparison of Salivary Beta Glucuronidase Activity in Chronic Periodontitis Patients with and without Diabetes Mellitus. Journal of Clinical and Diagnostic Research. 2014;8(6): ZC19-ZC21 doi: 10.7860/JCDR/2014/8713.4476
10. Kosoko AM, Olayanju OA, Rahamon SK and Arinola OG. Salivary lipid peroxidation and antioxidant status in nigerian cigarette smokers with or without periodontitis. Asian J Med Health 2017;3(3):1-9. DOI: 10.9734/AJMAH/2017/30749
11. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand. 1963;21:533-551. doi.org/10.3109/00016356309011240
12. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35. doi.org/10.3109/00016356408993968
13. Muhlemann HR, Son S. Gingival sulcus bleeding – a leading symptom in initial gingivitis. Helv Odontol Acta. 1971;15(2):107-113. PMID: 5315729
14. Russell AL. A system for classification and scoring for prevalence surveys of periodontal disease. J dent Res. 1956;35:350-359. DOI: 10.1177/00220345560350030401
15. Layik M, Yamalik N, Caglayan. Analysis of human gingival tissue and gingival crevicular fluid β glucuronidase activity in specific periodontal diseases. J Periodontol. 2000;71:618-24. DOI: 10.1902/jop.2000.71.4.618

16. Murray RK, Granner DK. Harper's Biochemistry. 23rd ed. Englewood Cliffs. New Jersey: Prentice Hall Inc. 1993;643.
17. Kaufman E, Lamster IB. Analysis of saliva for periodontal diagnosis- A review. J Clin Periodontol 2000;27:453-465. DOI: 10.1034/j.1600-051x.2000.027007453.x
18. Kinney JS, Morelli T, Brun T, et al. Saliva/pathogen biomarker signatures and periodontal disease progression. J Dent Res 2011;90:752-758. doi: 10.1177/0022034511399908
19. Lamster IB, Vogel Hartley. Lactate dehydrogenase, beta glucuronidase and arylsulphatase activity in GCF associated with experimental gingivitis in man. J Periodontol. 1985; 56:139-47. DOI:10.1902/jop.1985.56.3.139
20. Masakazu Nakamura and Jorgen Slots. Salivary enzyme origin and relationship to periodontal disease. J Periodont Res. 1983;18:559-69. DOI:10.1111/j.1600-0765.1983.tb00393.x
21. SK Balaji. Beta Glucuronidase Activity in Gingival Crevicular Fluid and Subgingival Microflora in patients with and without Adult Periodontitis. Asian J Exp Biol Sci. 2012; 3 (1): 142-55.
22. Lamster IB, Holmes LG, Gross. The relationship of β glucuronidase activity in crevicular fluid to probing attachment loss in patients with adult periodontitis. J Clin Periodontol. 1995; 22:36-44. DOI:10.1111/j.1600-051x.1995.tb01768.x
23. Ali SA, Telgi RL, Tirth A, Tantry IQ, Aleem A. Lactate Dehydrogenase and β-Glucuronidase as Salivary Biochemical Markers of Periodontitis Among Smokers and Non-Smokers. Sultan Qaboos University Med J, August 2018;18(3):e318-323. doi: 10.18295/squmj.2018.18.03.009
24. Pabst MJ et al. Inhibition of neutrophil and monocyte defensive functions by nicotine. J Periodontol. 1995;66(12):1047-55. DOI:10.1902/jop.1995.66.12.1047
25. Proye M, Caton J, and Poison A. Initial healing of periodontal pockets after a single episode of root planing monitored by controlled probing forces. J Periodontol.1982;53(5):296-301 DOI:10.1902/jop.1982.53.5.296