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The Effect of Systemic Doxycycline and Mechanical Therapy on GCF β -Glucuronidase Leves in Chronic Periodontitis Patients

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ORIGINAL ARTICLE

The Effect of Systemic Doxycycline and Mechanical Therapy on GCF β -Glucuronidase Leves in Chronic Periodontitis Patients

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ABSTRACT

The goal of periodontal therapy includes management of bacterial pathogens to control the destruction of the periodontal tissue. A number of different nonsurgical and surgical therapies have been used in achieving this goal. **Objective:** To evaluate the effectiveness of doxycycline compared with nonsurgical therapy alone or in combination with nonsurgical therapy on gingival crevicular fluid β -Glucuronidase levels (β G) and clinical parameters over a 20-week period in patients with chronic periodontitis. **Methods:** An interventional study comprising 60 patients with chronic periodontitis. They were divided into three groups of different treatment approach. The plaque index, gingival index, probing depth, and clinical attachment levels were recorded, and gingival crevicular fluid (GCF) samples were collected at 2, 6, 10, and 20 weeks, after treatment. **Results:** The levels of GCF β G and clinical parameters were higher when the treatment was initiated, and the levels decreased after treatment. No significant difference was found in the β G level when nonsurgical therapy and doxycycline were used alone. In comparison, when doxycycline and nonsurgical therapy were combined, the level of β G decreased significantly. **Conclusion:** The present study indicated the usefulness of doxycycline as an adjunct to nonsurgical periodontal therapy in lowering GFC β G levels and clinical parameters.

Keywords: *doxycycline; periodontitis; β -Glucuronidase; gingival crevicular fluid; nonsurgical periodontal therapy*

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INTRODUCTION

Periodontitis is a disease characterized by the loss of connective tissue attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium.¹ The microbial nature of many periodontal diseases has been recognized for a long time. More recently, it has been realized that host-related factors may be the keys for understanding the disease processes. Periodontal disease progression is episodic in nature on the tooth-site level; however, the risk of developing periodontal disease is principally patient based rather than site based.²

Bacterial virulence factors either result directly in degradation of host tissues or cause the release of biologic mediators from host tissue cells that lead to host tissue destruction. The mediators that are produced as a part of the host response that contributes to tissue destruction include proteinases, cytokines, and prostaglandins, as well as a variety of enzymes produced by periodontal microorganisms that cause tissue destruction.¹

The early diagnosis and treatment of progressive periodontitis is important because of the irreversible nature of this disease.³ Traditional clinical measurements (probes of the pocket depth, blood loss

upon probing, clinical attachment loss, plaque index, and radiographs) used for periodontal diagnosis are often of limited usefulness in that they are indicators of previous periodontal disease rather than present disease activity.⁴ New diagnostic tests need to be developed 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.⁵

β glucuronidase (β G) is a lysosomal enzyme that degrades proteoglycans and ground substance and are released from the azurophilic (primary) granules of PMNs in response to stimuli such as N-formyl-methionyl-leucyl-phenylalanine, platelet-activating factor, anaphylotoxin C5a, LTB₄, and IL8⁻ serves as a marker in periodontitis.^{6,7} β glucuronidase is a glycoprotein of about 332,000 Dalton.⁶⁻⁸ It is a homotetramer comprised of four identical subunits. β G, together with hyaluronidase, is involved in the catabolism of proteoglycans. The endoglycosidase hyaluronidase cleaves hexosaminidic linkages, producing tetrasaccharides. This tetrasaccharide is further degraded by β G and β -N-acetylhexosaminidase. Therefore, β G most likely contributes to non-collagenous matrix degradation in periodontal diseases.⁸

The relationship between β G activity and periodontal disease has clearly been shown.⁸ Furthermore, β G activity may be a good indicator or predictor of periodontal disease activity, and its potential for indicating primary granular release from PMNs has been observed. It has high sensitivity and specificity when related to occurrence of clinical attachment loss. This enzyme has also proved to be a good predictor of the response to treatment and the risk for future periodontal breakdown.⁹ Increased levels of β G in GCF have been associated with increased risk for periodontal attachment loss in systemically healthy individuals and are strongly correlated with probing depth (PD). However, decreased levels of β G in GCF have been linked with conservative periodontal therapy.^{2,10}

The essential goal of periodontal therapy is the successful management of bacterial pathogens to the extent that destruction of the periodontium is arrested. A number of different nonsurgical and surgical therapies have been successful in achieving this goal.¹¹⁻¹³ The primary nonsurgical approach involves mechanical scaling and root planing (SRP). The beneficial effects of SRP arise from a reduction in the microbial burden in the periodontal pocket, or a shift toward less pathogenic microflora.¹⁴ However, the efficacy of SRP may be compromised at tooth sites with deep periodontal pockets. Furthermore, the long-

term success of SRP may be affected by the remaining bacterial virulence factors and ineffective personal plaque control.¹²

Several antimicrobial agents have been attempted systemically as an adjunct to the mechanical treatment of periodontal disease.¹⁵ Systemic antibiotics may be used to reduce the bacterial load and to enhance the host's defense to infectious pathogens in chronic periodontitis. Tetracycline, metronidazole, and amoxicillin are among the antibiotics used in the treatment of periodontal disease, and their use is determined based on a patient's medical and dental history, disease diagnosis, and the possible side effects. Also, doxycycline is a potentially valuable antibiotic, with a broad spectrum of activity. Doxycycline has the capability of conditioning dentine and also enables fibrin linkage, both of which favor the formation of new attachment.^{16,17} Doxycycline potentiates osseous regeneration in periodontal defects due to its anti-collagenase activity.¹⁸

The purpose of this interventional study was to evaluate the efficacy of systemic doxycycline alone or in combination with nonsurgical periodontal therapy compared with nonsurgical periodontal therapy and the impact on the GCF β -glucuronidase levels and clinical parameters of patients with similar chronic periodontitis severity.¹⁹

METHODS

Study design

The present study was conducted in the Department of Periodontics, Dr Z. A. Dental College, AMU, Aligarh. The Institutional Ethics Committee of the Faculty of Medicine provided approval for the study. This was a randomized, interventional study in subjects with chronic periodontitis. The clinical trial extended over a 20-week period and was designed as a double-blind study.

Study population

A total of 60 untreated chronic adult periodontitis patients (consisting of 22 males and 38 females), aged 25 and over, were recruited from new referrals to the OPD of Department of Periodontics and Community Dentistry, Dr. Z. A. Dental College and Hospital, A.M.U., Aligarh. Informed consent was obtained from all the patients.

Inclusion criteria

The subjects included in this study were having no history of systemic disorders/smoking, no history of antibiotics and anti-inflammatory drugs within the past 6 months, and no history of any dental treatment within the past 6 months. The subjects must have at least 20 teeth remaining in the mouth and at least had five sites with > 4mm pocket depth. Following a baseline

Table 1. Clinical parameters measurement of all groups of treatment

| GROUPS | TIME (weeks) | | | | |
|---------------------------------------|--------------|-------------|-------------|-------------|--------------|
| | BASELINE | 2 | 6 | 10 | 20 |
| Plaque Index | | | | | |
| 1 | 1.36 ± 0.91 | 0.87 ± 0.13 | 0.78 ± 0.08 | 0.68 ± 0.35 | 0.79 ± 0.25 |
| 2 | 1.65 ± 0.18 | 1.43 ± 0.26 | 1.4 ± 0.25 | 1.34 ± 0.31 | 1.64 ± 0.122 |
| 3 | 1.64 ± 0.16 | 0.73 ± 0.11 | 0.65 ± 0.18 | 0.55 ± 0.18 | 0.56 ± 0.25 |
| Gingival Index | | | | | |
| 1 | 1.63 ± 0.26 | 0.91 ± 0.22 | 0.79 ± 0.19 | 0.76 ± 0.19 | 0.77 ± 0.18 |
| 2 | 1.63 ± 0.29 | 0.63 ± 0.19 | 0.57 ± 0.21 | 0.74 ± 0.18 | 1.45 ± 0.34 |
| 3 | 1.49 ± 0.25 | 0.67 ± 0.16 | 0.61 ± 0.15 | 0.53 ± 0.17 | 0.60 ± 0.19 |
| Probing Depth (mm) | | | | | |
| 1 | 4.69 ± 0.53 | 2.4 ± 0.6 | 1.09 ± 0.3 | 0.89 ± 0.27 | 0.75 ± 0.47 |
| 2 | 3.91 ± 0.65 | 3.46 ± 0.83 | 3.37 ± 0.74 | 3.48 ± 0.69 | 3.78 ± 1.14 |
| 3 | 3.93 ± 0.92 | 1.79 ± 0.85 | 1.27 ± 0.21 | 0.99 ± 0.19 | 1.09 ± 0.44 |
| Clinical Attachment Level (mm) | | | | | |
| 1 | 6.63 ± 0.35 | 3.43 ± 0.25 | 2.91 ± 0.17 | 2.85 ± 0.13 | 2.45 ± 0.31 |
| 2 | 6.34 ± 0.19 | 5.28 ± 0.51 | 4.88 ± 0.43 | 4.92 ± 0.47 | 5.2 ± 0.80 |
| 3 | 5.31 ± 0.46 | 3.34 ± 0.55 | 3.14 ± 0.28 | 2.77 ± 0.37 | 2.96 ± 0.38 |

Group 1 = scaling and root planing; Group 2= systemic doxycycline; Group 3= systemic doxycycline with scaling and root planing. Differences of the clinical parameters measurement are shown in in mean ± SD.

examination, the patients were randomly distributed into three groups of 20 members each. The patients in the first group were meticulously treated by SRP. The second group received systemically administered doxycycline from the first appointment at a dosage of 100 mg/ day for 14 days, with a loading dose of 200 mg on the first day. The third group of patients received systemically administered doxycycline from the first appointment at a dosage of 100mg/ day for 14 days, with a loading dose of 200mg on the first day along with SRP.

Treatment protocol

All the patients included in the study received oral hygiene instructions. The patients with periodontitis in groups 1 and 3 received nonsurgical periodontal treatment, which comprised instructions about oral hygiene and supragingival and subgingival debridement (SRP). The treatment took, on average, four sessions of 40 min for 2 weeks.²⁰ The SRP was performed with manual instruments (Gracey Curettes) and a Biosonic® ultrasonic dental scaler. Reevaluations were performed after 2, 6, 10, and 20 weeks, and oral hygiene reinforcement was done at each recall visit.

Experimental parameters

Determination of periodontal status

To determine the clinical periodontal status, probing depths (PD) and the clinical attachment level (CAL) were measured, and the plaque index (PI) and gingival index (GI) scores were recorded.^{21,22} These data were taken from the whole mouth for all teeth at four sites,

excluding third molars at the baseline and after 2, 6, 10, and 20 weeks.

Collection of GCF

The sites selected randomly were air-dried and isolated with cotton rolls to exclude contamination with saliva, and the crown of the tooth was gently cleaned to remove supragingival plaque. The GCF was collected by inserting absorbent paper points into the gingival sulcus for 30s, avoiding any bleeding from the marginal gingiva.²³ The paper points that were visibly contaminated with blood were discarded. To eliminate the risk of evaporation, the paper points were placed immediately in 1.5ml Eppendorf vials containing a buffer solution (potassium acetate buffer solution) and stored at -20°C.²³ The GCF samples were collected at the baseline and after 2, 6, 10, and 20 weeks from all the groups. All the samples were collectively analyzed for β-glucuronidase activity.

Laboratory studies

Estimation of βG concentration in the GCF: The βG concentration in the GCF was estimated by using the reagent kit supplied by Sigma Aldrich diagnostics®, and an spectrophotometric analysis was done using an autoanalyzer. The method used was based upon the modified procedure.^{24,25} A phenolphthalein standard curve was constructed into 10 concentrations of phenolphthalein ranging from 100 to 10 µg and were plotted against absorbance at 540nm (Figure 1) βG acts on phenolphthalein mono β glucuronic acid, liberating the free phenolphthalein. The rate of hydrolysis of

Table 2. The level of β -glucuronidase in gingival crevicular fluid of all groups of treatment

| GROUPS | TIME (weeks) | | | | |
|--|------------------|------------------|-----------------|-------------------|-------------------|
| | BASELINE | 2 | 6 | 10 | 20 |
| Level of β-glucuronidase in GCF (IU) | | | | | |
| 1 | 1480 \pm 97.97 | 980 \pm 2259.1 | 350 \pm 48.1 | 955 \pm 399.9 | 1105 \pm 1146.7 |
| 2 | 1780 \pm 258.1 | 400 \pm 779.7 | 225 \pm 408.5 | 1590 \pm 1093.5 | 1250 \pm 59.5 |
| 3 | 1780 \pm 260 | 480 \pm 1122.3 | 0 \pm 0 | 1135 \pm 544.3 | 1780 \pm 160 |

Group 1= scaling and root planing; Group 2= systemic doxycycline; Group 3= systemic doxycycline with scaling and root planing. Differences of the clinical parameters measurement are shown in in mean \pm SD.

phenolphthalein glucuronidase serves to assay the concentration of β G.⁹ The liberated phenolphthalein is estimated by the red color it gives at alkaline pH. Phenolphthalein glucuronide has hardly any absorption at the same pH.⁹

Statistical Analysis

The statistical test performed to analyze the results was a Student’s t-test since all samples had a normal distribution. A Student’s t-test for independent variables was applied to compare groups 1, 2, and 3. The differences within groups were analyzed by a one-way analysis of variance (ANOVA). When the difference was significant, the groups were analyzed by Tukeys HSD test bilaterally. The level of statistical significance was established ($p \leq 0.05$). The means and standard deviations were given to describe values.

RESULTS

Clinical Findings

The mean values of clinical parameters for all the groups at baseline and 2, 6, 10, and 20 weeks are shown in Table 1. The periodontal condition of groups 1, 2, and 3 markedly improved between the baseline and the recall visits at 2, 6, 10, and 20 weeks ($p \leq 0.001$). However, the SRP + doxycycline group showed a significantly greater reduction in PD and gain in the CAL when compared with the SRP alone and doxycycline alone groups at 10 at 20 weeks ($p \leq 0.001$, $p \leq 0.05$, and $p \leq 0.05$, respectively). A significant reduction in the PI & GI scores of all groups ($p \leq 0.001$) was observed, with significant differences detectable between them over the entire study period except in group 2, in which doxycycline alone was used ($p \geq 0.05$) at 20 weeks.

Laboratory Findings

The mean β G levels in the GCF for all groups for the entire study period are given in Table 2. All the groups had a similar GCF β G level at the baseline ($p \geq 0.05$). When SRP alone was used, the GCF β G levels were reduced significantly at the 2 and 6 week recall visits ($p \leq 0.05$), with no significant reduction at 10 and 20 weeks. When doxycycline alone was used, the GCF β G level was reduced significantly at 2, 6, and 10 weeks

($p \leq 0.01$) and was not significant at 20 weeks ($p \geq 0.05$). However, when SRP + doxycycline were used, the GCF β G level decreased significantly over the entire study period ($p \leq 0.001$). In Table 2, when comparing groups 1 (SRP alone) and 2 (Doxycycline alone), the GCF β G level was reduced significantly at 2, 6, and 10 weeks ($p \leq 0.05$) and was not significant at 20 weeks. When the SRP alone and doxycycline alone groups were compared with the SRP + doxycycline group, the GCF β G level was reduced significantly over the entire recall visit period ($p \leq 0.05$ and $p \leq 0.001$, respectively).

DISCUSSION

The present study was designed to evaluate the efficacy of doxycycline as an adjunct to SRP in chronic periodontitis patients. GCF has a widely accepted diagnostic potential. Although the GCF analysis performed on absorbent paper points is a frequently used method for evaluation, future studies should be standardized and a consensus should be reached on the methodological details, including data presentation.¹⁰ The samples collected for this study were kept at -20°C . The freezing time for all the samples in this study was similar (collected at the baseline and 2, 6, 10, and 20 weeks), and the evaluation of the samples therefore produced meaningful results. However, certain limitations could not be overcome, such as the choice of collection device, length of collection time, number of sample repetitions.²⁶⁻²⁸ It is a known fact that the amount of GCF collected varies from site to site, and hence the volume collected differs. In this study, we addressed this issue by standardizing the time of collection to 30 seconds, yet the volume of fluid collected differed.

The study presented significant improvements in clinical parameters in all the experimental groups. At the end of 2, 6, 10, and 20 weeks, this improvement was maintained mainly because of the efficient SRP and proper compliance. SRP therapy causes a resolution of inflammatory response and a cessation of the progression of periodontal disease and hence results in a reduction of pocket depth and a relative gain in clinical attachment.¹¹ Following doxycycline alone,

relatively no significant reduction was observed in the clinical parameters at 20 weeks. This may be due to several factors that are associated with the failure of systemic antibiotics in the treatment of periodontitis. These factors include patient compliance, incorrect dosage or duration of the agent, poor absorption, or poor distribution of the agent in the patient. In the present study, patient compliance and improper dosage were ruled out, as the patients were reinforced with proper oral hygiene instructions on every recall visit.

The result of the present study showed that the use of doxycycline in combination with SRP in chronic periodontitis patients provided a better clinical improvement ($p \leq 0.001$) beyond that obtained by SRP therapy alone or doxycycline alone. Previous studies have documented the effectiveness of systemic doxycycline on clinical parameters and on subgingival microbiota.²⁹ Doxycycline has several advantages over other drugs of its group. These include low dosage frequency, prolonged serum half life, the decreased effect of food on absorption, and decreased gastrointestinal effects.³⁰ It is a potent metalloproteinase inhibitor.³¹ Hence, the improvement in clinical parameters in group 4 is not only associated with the effectiveness of nonsurgical periodontal therapy but also to the beneficial effect of doxycycline.

The interpretations of the present study with regards to GCF β G levels were 1) that the systemic doxycycline therapy alone seemed to result in no or minimal effect on β G levels as compared to SRP and 2) that the positive effects in the SRP + doxycycline-treated quadrants, beyond those achieved with traditional SRP, could be ascribed to the adjuvant effect of drug therapy. To our knowledge, comprehensive studies of changes in β G levels during and after the systemic administration of antibiotics have not been performed.

The findings of the present study confirmed the relationship between β G and periodontal disease, since increase enzyme activity in GCF was observed when clinical periodontal destruction was present. This is in accordance with three previous studies.^{23,33,34} Our data further show that when GCF was analyzed for the presence and levels of β G activity, the presence of detectable levels of β G was shown in all the subjects. However, the diseased patients at the baseline presented higher enzyme activity than the periodontally treated patients. Therefore, these findings support the previous studies, suggesting a role for increased host-originated β G in the pathogenesis of periodontal disease.^{10,35}

As indicated in previous studies conducted on various GCF components, including enzymes, the highest GCF β G activity was found in patients with disease when no treatment was initiated.^{10,31-35} This increase in β G can be attributed to the hyperactive state and pronounced response of PMN as a consequence of the severity of

microbial virulence factors and also to the lytic effect of more pathogenic subgingival bacteria on host cells leading to an intense host enzyme release.³⁶

The present investigation demonstrated that the β G levels in chronic periodontitis patients in the absence of any treatment were significantly raised as compared to those in patients receiving treatment. This is in agreement with the findings who reported that a comparison of the baseline and 2-week post-therapy data for the entire population of his study indicated that conservative periodontal therapy tended to decrease β G levels in the GCF.³² This study is also in accordance with the study that examined the effect of SRP on the levels of β G in GCF over a 24-week period and showed that β G significantly decreased following SRP.³⁵ In the present study, following SRP, the β G levels decreased significantly at 6 weeks ($p \leq 0.01$), but not at 10 and 20 weeks. This may be due to poor patient compliance in spite of repeated reinforcements.

In the present study, after SRP + doxycycline, the GCF β G levels showed a marked reduction throughout the study period. This reduction was highly significant ($p \leq 0.001$) when compared with SRP alone or doxycycline alone. The decreased enzyme levels may be due to the nonsurgical periodontal therapy (SRP and oral hygiene instructions), which causes a reduction in bacterial load in the periodontal environment.¹¹ Also, the added effect of doxycycline in downregulating the host collagenases, thereby reducing the enzyme levels, may account for the decreased GCF β G levels.¹⁷ This study demonstrated that the GCF β G levels in the only SRP and only doxycycline groups tended to increase at 10 and 20 weeks compared to the levels at 6 weeks, whereas the GCF β G levels in the SRP + doxycycline group continued to decrease over 20 weeks. This indicates the usefulness of doxycycline as an adjunct to nonsurgical periodontal therapy over an extended period of time.

It identified that β G as a potential diagnostic marker of periodontitis was present in GCF.¹ It showed relatively elevated levels of β G in GCF to various thresholds of increased probing depth or probing attachment loss.³⁶ The relative risk of disease progression when elevated levels of the enzyme were present ranged from 6 to 14 times. Their analysis reported data for the patients, but not for the individual sites. Nevertheless, our study emphasizes the association of a prominent influx of PMN cells into the crevice (as a measure of the intensity of the acute inflammatory response) to destructive periodontitis. Though the biochemical analysis of β G holds promise for the early detection of periodontal disease and the evaluation of treatment outcome, further studies must determine the longitudinal relationship of GCF β G activity to disease progression.

CONCLUSION

The findings of the present study confirm the usefulness of doxycycline as an adjunct to nonsurgical therapy in reducing GCF β G levels and the clinical parameters. Our results demonstrate a correlation between GCF β G activity and periodontal clinical parameters. The study demonstrates the role of β G in the progression of periodontal disease and in determining the outcome of treatment.

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