Effect of 1% curcumin gel on myeloperoxidase activity in GCF and periodontal status in the initial phase of orthodontic tooth movement

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Abstract

AIM: To explore the potential effect of locally applied 1% Curcumin on myeloperoxidase (MPO) enzymatic activity in gingival crevicular fluid (GCF) and on the periodontal status during the initial phase of orthodontic tooth movement.

SETTINGS AND DESIGN: Forty patients (26 females and 14 males) aged 12-25 years who required fixed orthodontic treatment were randomly divided into two equal groups. The control and test groups were similar in the various baseline parameters, including standard oral hygiene protocol. Moreover, 1% Curcumin gel was applied around mandibular anterior teeth in the test group twice daily, from three days before to 14 days after the placement of archwires. MPO activity and periodontal status were recorded at five different time points; before placement of archwire (baseline), immediately after placement of archwire, 2 hours, 7 days, and 14 days later.

STATISTICAL ANALYSIS USED: The data were analyzed using paired t-test for intragroup differences and the unpaired t-test for intergroup differences at five different time points. Statistical significance in the intragroup and intergroup difference of Plaque and Gingival index was calculated using the unpaired t-test.

RESULTS: Maximum MPO enzymatic activity in GCF was observed two hours after the placement of the archwire. MPO activity decreased slightly on the seventh day, but values were still elevated as compared to baseline. However, MPO activity came back to the values similar to baseline on day 14 in the control group and significantly lower than the baseline in the test group. The inter-group differences in clinical periodontal parameters were non-significant.

CONCLUSIONS: The locally applied 1% Curcumin gel appears to decrease the MPO activity in GCF on the 14th day after placement of the archwires. However, clinical periodontal status in the initial phase of tooth movement is unaffected by curcumin if patients adhere to good plaque control.

Keywords: Curcumin, gingival crevicular fluid, inflammation, myeloperoxidase, orthodontic tooth movement

Introduction

Application of forces for orthodontic tooth movement results in remodeling of the periodontal tissues.⁴ This process comprises local synthesis and release of different cytokines, leucocytes, and enzymes.⁴ The resulting inflammation response is characterized by neutrophilic infiltration. Cytokine-mediated stimulation of neutrophils releases the myeloperoxidase (MPO) enzyme, which is a potent oxidant and can also lead to host cell and tissue damage.⁵ The estimation of MPO enzymatic activity in gingival crevicular fluid (GCF) is a reliable indicator of periodontal inflammation.⁶⁻⁸ Inflammatory

How to cite this article: Samita, Verma SK, Sharma VK, Moinuddin, Ahad A. Effect of 1% Curcumin gel on Myeloperoxidase activity in GCF and periodontal status in the initial phase of orthodontic tooth movement. J Orthodont Sci 2022;11:55.
response can be estimated indirectly by assessing biological markers in GCF, in order to prevent the adverse effect on the tooth and supporting structures.\[^{8,9}\]

Furthermore, orthodontic appliances are often associated with poor oral hygiene and gingival inflammation. Endogenous molecules thus released may affect the rate of tooth movement. Therefore, a need arises for an anti-inflammatory and anti-plaque agent that could treat the symptoms effectively without adversely affecting the rate of tooth movement.

Turmeric has been used both locally and systemically for various inflammatory conditions. This herb contains a group of compounds known as curcuminoids, composed of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the principal curcuminoid and comprises approximately 77% of turmeric.\[^{10}\] Curcumin acts as a potent antioxidant, anti-inflammatory, and antimicrobial agent that affects various organ systems. The application of turmeric as a locally delivered drug prevents plaque formation and gingivitis.\[^{11,12}\]

Aims of the study: Primary aim was to explore the potential effect of the local application of 1% Curcumin gel on the MPO activity in GCF, and the secondary aim was to evaluate the effect of 1% Curcumin on clinical periodontal parameters during the initial phase of orthodontic tooth movement.

The null hypothesis proposed that there is no potential effect of local application of Curcumin gel on the MPO activity in GCF.

### Subjects and Methods

The prospective study involved the patients receiving fixed orthodontic treatment in the Department of Orthodontics and Dentofacial Orthopaedics. The study was approved by the Institutional Ethics and Research Advisory Committee (R. No. 710, Ortho/DC 16) on 23\(^{rd}\) February 2015 and was conducted from March 2015 to December 2016 according to the principles outlined in the Declaration of Helsinki (1975) on experimentation involving humans as revised in 2013.

### Sample size estimation

The sample size was calculated after the effect size estimation of MPO activity using the OpenEpi software. Using the variance statistical test with $\alpha = 0.05$, $\beta$ (power) = 80% and CI = 0.95, a calculated effect size of 7.6 was input. The sample size was calculated to be 20 for the control group and 20 for the test group. The total sample size was calculated to be 40.

A total of 125 patients with crowding in the mandibular anterior region were screened as potential participants. Based on the inclusion and exclusion criteria, and willingness to sign the informed consent, 40 subjects were included in the study. An outline of the sample selection has been presented in Figure 1. Inclusion criteria involved systemically healthy patients with crowding in mandibular anterior teeth, age group of 12-25 years, and no history of previous orthodontic treatment. Exclusion criteria included mean probing depths >3 mm in any of the mandibular anterior teeth, gingival recession, trauma from occlusion, history of local or systemic antibiotics or anti-inflammatory drugs in the last three months, history of chronic systemic illness, and smoking.

Patients were instructed to follow the standard oral hygiene protocol. All of them were subjected to full-mouth scaling and root planing using ultrasonic scalers and curettes. Tooth polishing was also performed with a prophylaxis paste to minimize the roughness and subsequent plaque accumulation. All included participants were trained in the sulcular brushing technique and dental floss use to ensure excellent plaque control. Two weeks later, they were followed for adherence to oral hygiene instructions. Bonding was not performed until the patients showed a mean Plaque Index (PI) < 0.5. Patients were divided randomly into control ($n = 20$) and test ($n = 20$) groups. The amount of crowding in mandibular anterior teeth was recorded for each patient based on model analysis.

Control and test groups were similar in baseline parameters including standard oral hygiene protocol. Moreover, patients in the test group were asked to apply 1% Curcumin gel (Curenext Oral Gel, Abbott India Ltd) over the gingiva of mandibular anterior teeth, twice daily after brushing their teeth in the morning and the night. Curcumin application continued from three days before bonding to 14 days after bonding. Patients were evaluated for adherence to oral hygiene instructions at every visit. Clinical parameters of periodontal status,
i.e., Gingival index (GI) and Plaque index (PI) were recorded using a set of mouth mirror, explorer, and UNC-15 periodontal probe (Hu-Friedy, Chicago, IL, USA) at five different time points before and during initial orthodontic activation; T0, T1, T2, T3, and T4. GCF collection for the estimation of MPO enzymatic activity was also performed at each of these five visits. All the data were collected by two examiners who were not aware of the group allocation.

T0 (Baseline): Patients were examined two days before bonding and placement of archwires,

T1: Immediately after the bonding and placement of archwires,

T2: Two hours after, the bonding and placement of archwires,

T3: Seven days after, the bonding and placement of archwires,

T4: Fourteen days after, the bonding and placement of archwires.

The initial activation was done using MBT 0.022” × 0.028” inch slot appliance. The first archwire for all patients was a 0.012-inch NiTi alloy wire placed with elastomeric ligatures in each bracket.

Collection of GCF samples and estimation of MPO activity
Gingival crevices were dried after isolation of mandibular anterior teeth using cotton rolls. GCF samples were taken with the PerioPaper® strips (Oraflow Inc. New York, USA) from the mesiolabial and distolabial aspects of all the mandibular anterior teeth. The samples were ensured not to be contaminated with saliva or blood. The filled strips were placed individually with 100 μL buffer in sterile Eppendorf tubes. For 10 s at 4°C, these were centrifuged at 13,000 g. Until further analysis, supernatants were collected and stored at -70°C. MPO enzymatic activity was assessed using the MPO calorimetric assay kit (Catalog No. MAK068, Sigma Aldrich, St.Louis, USA). The 99 well plates were analysed by a spectrophotometer. MPO activity was calculated by the following equation:

\[
\text{MPO Activity} = 5B \times \text{Sample Dilution Factor} \div (\text{Reaction Time}) \times V.
\]

In this equation, B was the Amount (in mole) of TNB (Tris-NaCl-blocking buffer) consumed, and V referred to the sample volume (mL) added to the well.

Statistical analysis
For statistical analysis, the average value of the MPO enzymatic activity from the mesiolabial and distolabial samples of all mandibular anterior teeth was finally taken. Statistical significance was calculated with the paired t-test for intra-group differences and the unpaired t-test for intergroup differences at five different time points. Statistical significance in the intragroup and intergroup difference of PI and GI was calculated using the unpaired t-test.

Results
A total of 40 patients were included in this study, and there were no drop-outs. Twenty-six out of them (65%) were females, with equal numbers (13 each) in both groups. No adverse effect of curcumin was reported by any patient in the test group. Demographic, periodontal, and crowding parameters had no statistically significant intergroup difference [Table 1] at baseline (T0). The primary outcome parameter of the study was the MPO enzymatic activity (milliunits/mL), while the secondary outcome parameters were PI and GI.

Comparison of MPO enzymatic activity
In both control and test groups, the mean MPO enzymatic activity increased from T0 to T1, T1 to T2, and then decreased from T2 to T3 and T3 to T4. Peaks in the MPO enzyme activity were observed at T2 in the control group (24.62 ± 2.92) as well as in the test group (23.51 ± 3.40) [Table 2]. MPO values in the control group were higher at all five-time points compared to the test group. However, the difference was statistically significant only at T4 [Figure 2, Table 3].

Comparison of periodontal parameters
Intragroup, as well as the intergroup comparison of PI and GI, showed no significant difference in the mean values at any time point [Table 4].

The null hypothesis is rejected in this study as there was a significant effect of local application of 1% Curcumin gel on the MPO activity in GCF.

Discussion
Periodontal inflammation and orthodontic tooth movement are known to influence each other.[15] Initial orthodontic tooth movement results in an inflammatory response, where cytokine-mediated stimulation
can provide an accurate insight into the severity of gingival inflammation around a tooth.\textsuperscript{[19]}

Patients with mandibular anterior crowding were included in the present study, which is observed in most of the patients undergoing orthodontic treatment irrespective of the associated malocclusion. Kaur et al.\textsuperscript{[20]} recorded the prevalence of malocclusion in adolescents and found that crowding was present in 57.69\% of the subjects. Alignment of lower incisors results in correction of dental irregularities, reducing plaque accumulation, and improving gingival health.\textsuperscript{[21]} It has been reported that the degree of dental crowding itself does not affect the inflammatory status in the periodontium, during the initial stage of tooth movement.\textsuperscript{[19]}

The age group of the patients seeking orthodontic treatment usually falls in the range of 12-25 years. This age group was selected to clinically implicate the results of the present study on the majority of patients.

Table 3: Comparison of the mean MPO enzyme activity (milliunits/ml) between control group and test groups at T0, T1, T2, T3 and T4

| Time | Control Group | Test Group |
|------|---------------|------------|
| T0   | 16.35±1.59    | 16.38±1.69 |
| T1   | 16.98±1.44    | 16.90±1.75 |
| T2   | 24.66±2.92    | 23.51±3.40 |
| T3   | 21.06±3.02    | 20.66±3.05 |
| T4   | 15.98±1.69    | 11.15±1.60 |

\*Statistically significant. T0 (Baseline): Patients were examined two days before bonding and placement of archwires, T1: Immediately after the bonding and placement of archwires, T2: Two hours after the bonding and placement of archwires, T3: Seven days after the bonding and placement of archwires, T4: 14 days after the bonding and placement of archwires.

Figure 2: Line diagram showing Mean MPO enzymatic activity in control and the test groups over five different time points. T0 (Baseline): Patients were examined two days before bonding and placement of archwires, T1: Immediately after the bonding and placement of archwires, T2: After 2 hours, the bonding and placement of archwires, T3: After 7 days, the bonding and placement of archwires, T4: After 14 days, the bonding and placement of archwires.
Anatomical and functional changes in periodontium are associated with the aging process.\cite{22} Aging leads to biomolecular changes in the periodontium, which may be due to increased cell response to mechanical stress leading to cytokine secretion involving bone loss and systemic endocrine changes.\cite{23} To avoid the effect of periodontal changes associated with aging, only adolescents and young adults were included in the present study. The initial (alignment) phase of orthodontic treatment consists of the correction of irregular teeth\cite{24} and no force is applied before this phase. Therefore, any biomarker’s baseline activity due to orthodontic force can be assessed in this phase and compared with biomarker activity in subsequent stages. Strict oral hygiene was ensured in both groups to avoid it being a confounding factor, as evident in the baseline comparison of the PI and GI. Orthodontic treatment is commonly associated with generalized gingivitis.\cite{21} Locally applied drugs have been used along with standard oral hygiene instructions and scaling for plaque control and prevention or treatment of gingival inflammation. The Curcumin oral gel is a potent antioxidant and anti-inflammatory agent. It inhibits biosyntheses of both inflammatory cytokines and neutrophil functions.

GCF is produced by the extravasating of circulating plasma directly in the gingival sulcus.\cite{18} Although saliva contains biomarkers similar to GCF, it represents the entire oral environment rather than a specific tooth. The GCF is likely to reflect the local inflammation resulting from orthodontic movement, more accurately than saliva.\cite{19} GCF is useful for monitoring biochemical changes as it reflects local inflammatory response more accurately than other biological fluids.\cite{9} MPO enzyme is present in azurophilic granules of PMNs.\cite{23} Cao et al.\cite{6} reported that the number of PMNs in the periodontal tissues is directly correlated with the level of MPO activity. Therefore, MPO activity is a reliable indicator to determine the level of inflammation, as evident from previous studies.\cite{6,7}

First samples were taken at T0, i.e., two days before placing the fixed appliances, to establish baseline values for the enzyme. This data shows that curcumin which was being applied during the previous 24 hours in the test group, had no effect on a healthy periodontium devoid of any orthodontic intervention. It has been reported in an in vitro study that curcumin enhanced cellular resistance to oxidative damage after 18 hours of incubation with bovine aortic endothelial cells.\cite{26}

T1 samples were recorded immediately after placing the wire (within minutes), while T2 samples were taken two hours after activation. Increased MPO activity from T0 to T1 and T1 to T2 could be attributed to the inflammation that begins just after the application of forces.\cite{27} Proffit\cite{4} explained this process as the physiologic response to sustained pressure against a tooth, which is characterized by altered blood flow and oxygen tension, followed by the release of prostaglandins and cytokines. These biomolecules eventually affect cellular activity and change enzyme levels. Krishnan and Davidovitch\cite{28} stated that the acute inflammatory process is predominantly exudative, consisting of plasma cells and leukocytes during initial tooth movement.

T3 samples were taken on the seventh day after activation of the fixed appliance, and subsequently, T4 samples were taken on day 14. Mean MPO enzymatic activity in both groups decreased from T2 to T3 and then further decreased at T4 with values similar to T1. This finding may be due to the transformation of acute inflammation into a chronic one characterized by the proliferation of fibroblasts, endothelial cells, and osteoblasts. It continues until the next clinical activation of the appliance. During this phase, leukocytes still migrate into the periodontal tissues and modulate the process of remodeling.\cite{26}
Similar variations in enzymatic activity have been reported in many previous studies. Navarro-Palacios et al. estimated MPO levels in different irregularity groups i.e., minimum and severe crowding and reported similar results. They also found that the highest MPO activity was detected at two hours; with a slight decrease in the values on the seventh day and it reaches levels similar to the baseline on the 14th day. Marcaccini et al. also showed that MPO activity is increased significantly at two hours after force application in both GCF and saliva. However, they found it to return to baseline level within a week. They also reported that MPO levels decreased during the second week in both GCF and saliva. A decreased MPO activity was observed in the present study at T4 compared to T1 in both the groups; however, the difference was statistically significant only in the test group. It may be attributed to the anti-inflammatory effects of curcumin which has been widely reported in the literature. Studies based on chronic periodontitis patients have shown curcumin to be better as compared to 0.2% chlorhexidine gluconate and control (saline) as an adjunct to scaling and root planing. Behal et al. concluded that locally applied 2% whole turmeric gel and scaling and root planing (SRP) is more effective than SRP alone.

The anti-inflammatory activity of curcumin is achieved through many mechanisms. It inhibits the arachidonic acid pathway and reduces pro-inflammatory leukotriene synthesis. It is also a potent inhibitor of 5-hydroxyeicosatetraenoic acid and has been found to reduce neutrophil infiltration in inflammatory conditions. Oxygen scavenging activity of curcumin has also been reported. Although there were no clinical signs of gingival inflammation during two weeks of follow up in any group, the application of curcumin was able to reduce the indirect marker of inflammation, i.e., MPO activity in GCF, much below the baseline level.

The present study used a non-invasive, rapid, and a relatively simple method of GCF collection using PerioPaper® strips which is easily reproducible in future studies. This technique also has low chances of contamination with blood compared to the micropipettes, avoiding errors in the estimation of enzymatic activity. However, our experiment focused on the mandibular anterior teeth only, that too for a brief period. The anti-inflammatory activity of curcumin might have some effect on the tooth movement in the long term, which was not evaluated in the present study.

**Conclusion**

There was a significant increase in the MPO activity in the GCF of mandibular anterior teeth in both control and test groups during the first two hours after the application of orthodontic forces. The raised level of MPO activity continued for one week. In both control and test groups, the MPO activity decreased significantly after the first week. However, the MPO activity on 14th day was comparable to baseline values in the control group and significantly lower than baseline values in the test group. However, the curcumin failed to produce any significant change in the clinical parameters of gingival inflammation during 14 days of evaluation.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflict of interest.

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