

## Assessment of Myeloperoxidase Levels In Relation to the Nature and Duration of Orthodontic Forces – An In vivo, Split Mouth Study

Vineet Sharma<sup>1</sup>, Supriya Nambiar<sup>2</sup>, Poornima Manjrekar<sup>3</sup>,  
Asavari Desai<sup>4</sup> and Pramod Philip<sup>5</sup>

<sup>1</sup>Department of Orthodontics, Manipal College of Dental Sciences, Mangalore, India.

<sup>2</sup>Department of Orthodontics, Manipal College of Dental Sciences, Mangalore, India.

<sup>3</sup>Department of Biochemistry, Kasturba Medical College, Mangalore, India.

<sup>4</sup>Department of Orthodontics, Manipal College of Dental Sciences, Mangalore, India.

<sup>5</sup>Department of Orthodontics, Case Western Reserve University, Ohio, USA.

\*Corresponding author E-mail: nambiar.supriya@gmail.com

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To assess the change in the myeloperoxidase levels (MPO) in the gingival crevicular fluid (GCF) at different time intervals during the course of orthodontic treatment with the application of intermittent and continuous forces. It was an interventional, split mouth study done on twenty patients scheduled to undergo fixed orthodontic treatment with therapeutic extractions of the first premolars. Mini implant assisted enmasse retraction was done after leveling and aligning of the arch. Continuous force with the help of Niti coil spring was used in the first quadrant and intermittent force with an elastomeric chain was used in the second quadrant. MPO was collected from the GCF using Whatman's paper at baseline, 1 week and 2 weeks after the application of retraction forces. The levels were assessed using a commercially available ELISA kit. Mean MPO activity was increased in the GCF of all the patients at 1 and 2 weeks after retraction, in both quadrants. At 2 weeks, MPO levels at the intermittent force application site were found to be significantly higher than those at the site of continuous force application. Myeloperoxidase is a useful marker to assess inflammation associated with orthodontic tooth movement. Intermittent forces cause a higher degree of inflammation as compared to continuous forces.

**Keywords:** Myeloperoxidase, Orthodontics, Tooth.

Tooth movement by orthodontic force application is characterized by remodeling changes in dental and paradental tissues, including dental pulp, periodontal ligament (PDL), alveolar bone and gingiva. These tissues, when exposed to varying degrees of magnitude, frequency and duration of

mechanical loading, express extensive macroscopic and microscopic changes. Orthodontic tooth movement differs markedly from physiological tooth eruption which is a slow process that occurs mainly in the occlusal and mesial direction into cancellous bone. In contrast, orthodontic tooth

movement is characterized by the abrupt creation of compression and tension regions in the PDL and can occur rapidly or slowly depending on the characteristics of the applied force and the biological response of the PDL. These force-induced strains alter the PDL's vascularity and blood flow resulting in local synthesis and release of various key molecules such as neurotransmitters, cytokines, growth factors, colony-stimulating factors and arachidonic acid metabolites. These molecules evoke many cellular responses by various cell types in and around teeth, providing a favorable microenvironment for tissue deposition or resorption.<sup>1</sup>

Orthodontic tooth movement promotes remodeling of the alveolar bone, which is mediated by inflammatory-like reactions characterized by vascular changes and infiltration of leucocytes. During orthodontic tooth movement changes in the periodontium occur, depending on the magnitude, direction, and duration of the applied force. Biochemical analysis of the gingival crevicular fluid (GCF) is a useful and promising method for monitoring these changes at a single site during a certain period as well as investigating the response of dental and periodontal tissues to fixed orthodontic appliance forces.

Azurophilic granules of polymorphonuclear neutrophils (PMNs) contain the enzyme myeloperoxidase (MPO), which is included in the host-derived group of compounds that are important for the tissue defense promoted by PMNs. It has been established that the level of MPO activity is directly correlated with the number of PMNs in the tissues and therefore MPO activity, but not its protein amounts is used in studies on inflammation of tissues. Studies conducted previously have shown that MPO is increased in the GCF collected from inflamed sites.

Since MPO levels found in the GCF represent the extent of PMN infiltration, the determination of MPO will be a valuable tool to assess the degree of inflammation in tissues which is of particular significance in orthodontics. After the application of this force, PMNs migrate into the tissues and finally extravasate in increased numbers into the GCF.

Previous studies have found that MPO level is increased in patients undergoing fixed orthodontic<sup>2</sup> treatment but the characteristics of

the involved mechanical stress have not been specified.<sup>3</sup>

Therefore this study was done to investigate the changes of MPO levels in the gingival crevicular fluid with different types of force *in vivo*.

### Objectives

To assess the change in the myeloperoxidase levels in GCF at different time intervals during the course of orthodontic treatment with the application of intermittent and continuous forces.

## MATERIALS AND METHODS

It was an experimental, *in vivo* split mouth study for which clearance from the Institutional Ethics Committee, Manipal College of Dental Sciences, Mangalore dated 13<sup>th</sup> December, 2014 (Ref No: MCO198/2014) was obtained.

### Methods

Twenty orthodontic patients who were scheduled to undergo fixed appliance therapy were enrolled in the study with prior informed consent. Patients of either gender, requiring therapeutic extraction of the first premolars and were generally healthy were included in the study. Smokers and subjects who had been administered anti-inflammatory drugs/antibiotics in the month preceding the study or had periodontally compromised dentitions were not considered.

Fixed appliance therapy was started using brackets with an 0.022" slot and Roth prescription. Levelling and aligning was carried out conventionally after which space closure was initiated by en masse retraction using mini implants (SK Surgical, India) placed between the second premolar and first molar.

In order to compare the effect of continuous and intermittent forces on MPO levels, a Niti closed coil spring (6mm, Astradent, India) and closed clear elastomeric chain (Rabbit force, India) was used in the first and second quadrants respectively. A retraction force of five ounces, measured with a Dontrix gauge, was used on both sides.

GCF samples were taken from the mesiobuccal or distobuccal aspects of the canine using Whatman absorbent paper strips in both quadrants and were maintained separately in different vials. The GCF samples were taken at three time intervals namely,

T1- (Baseline sample)-taken before retraction was begun,

T2-After one week of retraction

T3-After two weeks of retraction.

Oral hygiene was maintained by oral prophylaxis and reinforcing instructions. Subjects were instructed not to consume anything for 1 hour before the examination. After isolating the tooth with a cotton roll, the crevicular site was gently dried with an air syringe. The GCF samples were collected by using Whatman absorbent paper strip placed into the sulcus or pocket until mild resistance was felt and left in place for 30 seconds (Figure 1). All the samples were always taken from the same site at the 3 time points. Strips contaminated by saliva or blood were excluded. At T1 and T2, the elastomeric chain placed in the left quadrant was reactivated and the force was calibrated to five ounces as described by H Park *et al.*<sup>4</sup>. The NiTi closed coil spring used on the right side was left as such. All strips with GCF were immediately and individually placed in Eppendorf vials containing 100 mL of buffer (50 m mol/L Tris-HCl, pH 7.4; 200 m mol/L NaCl; 10 m mol/L CaCl<sub>2</sub>; and 0.02% Triton X-100). The vials were maintained on ice for 30 minutes and then centrifuged at 13,000g for 10 minutes at 4 degree celsius (Figures 2). Supernatants were stored at -70 degree C until further analysis. All analyses were performed directly on this 100 mL sample, without further dilution.

The Myeloperoxidase levels were then estimated using commercially available ELISA kit (QAYEE BIO QY EO1364). It uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the level of Myeloperoxidase (MPO).



**Fig. 1.** GCF collection on the right side

The test was run by addition of standard, test sample and HRP-labeled Myeloperoxidase (MPO) antibodies to enzyme wells which were pre-coated with Myeloperoxidase (MPO) antibody, then the wells were incubated and washed to remove the uncombined enzyme. Upon adding chromogen solution A and B, the color of the liquid changed into blue, and the reaction with the acid caused the color to change to yellow. The depth of color and the concentration of the Myeloperoxidase (MPO) sample are positively correlated.

#### Statistical analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS version 20).

Students unpaired 't' test was performed to assess the correlation between right and left quadrants where light continuous forces and intermittent forces were applied respectively, at the three time intervals -T1, T2 and T3.

Analysis of variance (ANOVA) was used to determine whether there were any significant changes in either of the two sides over the duration of the study at T1, T2 and T3. The values of this procedure were derived using Fisher test.

To compare the changes of the right and left sides separately over the duration of the study at the mentioned timelines and to check whether there are any significant changes at different intervals, Bonferroni 't' test was carried out.

## RESULTS

Comparison of intragroup changes in MPO levels at the three timelines (Table 1) p values of <0.05 were considered as statistically significant.

At T1, the mean value of Myeloperoxidase for the right side (light continuous force) was



**Fig. 2.** GCF samples before cold centrifugation

found to be 298.85 ng/ml s.d 22.35, and the left side (intermittent force) it was 287 ng/ml s.d.24.09.

At T2, the mean value of Myeloperoxidase for the right side was found to be 346.65 ng/ml s.d.27.68 whereas for the left side it was 330.95 ng/ml s.d.34.75.

At T3, the mean value of Myeloperoxidase for the right side was found to be 325.05 ng/ml s.d.27.92 whereas for the left side it was 349.75 ng/ml s.d. 32.19.

Students 't' test revealed a statistically significant difference between right and left sides at T3 with a p value of 0.013. However, no significant difference was noted at T1 and T2 with p values of 0.115 and 0.122 respectively.

#### Comparison of MPO levels between the two quadrants at all the three timelines

The mean, standard deviation, Fisher test value and p values for the same are given in (Table 2, fig 3).

For the right side where continuous force was applied, the mean value of Myeloperoxidase at T1 was found to be 298.85 ng/ml 22.35. At T2 it was 346.65 ng/ml s.d.27.68 and T3 the mean was 325.05 ng/ml s.d. 27.92.

For the left side, the mean value of myeloperoxidase at T1 was 287.00 ng/ml s.d.24.09. At T2 the mean was 330.95 ng/ml s.d. 34.75 and at

T3 the mean value was 349.75 ng/ml s.d. 32.195.

Analysis of variance (ANOVA) revealed that the MPO levels for the right side at the three time levels was found to be highly significant at p value of <0.001. The Fisher value was 16.803.

Similarly the myeloperoxidase values for the left side at the three timelines was also found to be highly significant at p value of <0.001. The Fisher value was 22.026.

Comparison of MPO levels of the right and left quadrants at a particular time line and its relation to the other two timelines (Table 3).

For the right side, the comparison of myeloperoxidase values between T1 and T2 showed mean difference of 47.80 which was highly significant at p value of <0.001. The comparison between T1 and T3 showed a mean value of 26.20 which was statistically highly significant with a p value of 0.007. The comparison between T2 and T3 showed a mean difference 21.60 which was significant at p value of 0.34.

Similarly for the left side, the comparison of myeloperoxidase values between T1 and T2 showed mean difference of 43.95 which was highly significant with a p value of <0.001. The MPO levels between T1 and T3 showed mean difference of 62.75 which was also highly significant with a p value of <0.001. However, the difference between

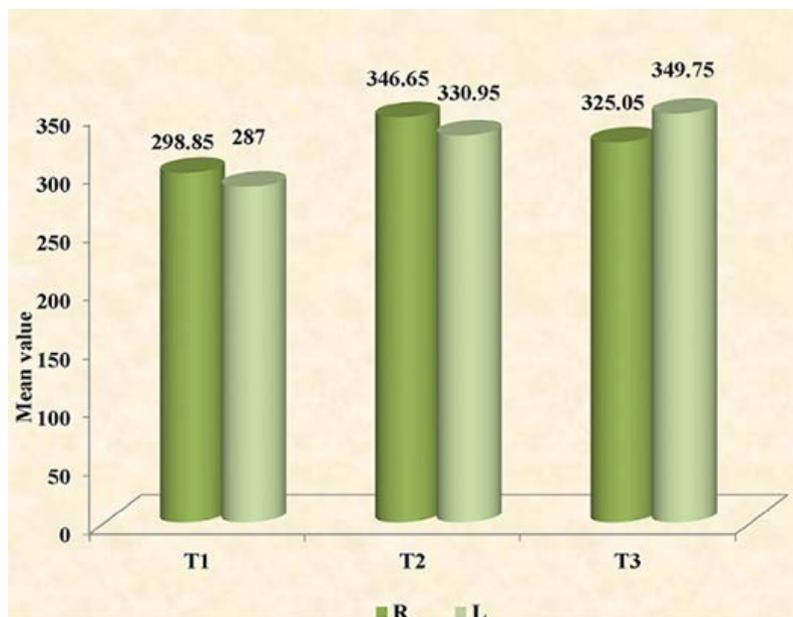


Fig. 3. Comparison of Myeloperoxidase levels between the two quadrants at different time intervals

T2 and T3 was not significant as the p value was 0.173.

## DISCUSSION

Judging the amount of inflammation after the application of orthodontic forces has always remained elusive for the orthodontist. There was always a need felt for some simple efficient technique which would help an orthodontist to gauge the amount of inflammation around a specific site. This is all the more important so as to avoid potential problems arising from excessive forces which could then only be seen after taking X-rays several months into the treatment.

Several studies in the past have been done to evaluate the level of inflammatory markers such as cytokines and proteases during orthodontic tooth movement.<sup>5,6,7</sup> In this study, we have attempted to measure the effect on Myeloperoxidase levels during enmasse retraction using intermittent force and light continuous force in different quadrants. A significant advantage of assessing myeloperoxidase levels over cytokines and proteases is that it can

be measured with a relatively simple assay which just uses substrate of the enzyme (MPO) and after a mere 30 minutes of reaction the readings can be obtained, so this could prove to be a valuable chairside tool in the days to come.

Due to the high sensitivity and specificity required for the detection of myeloperoxidase in the sparse quantity of GCF available for the study, ELISA was thought to be more reliable method for estimation as opposed to manual method which has been used for samples having relatively larger volume such as plasma and serum. ELISA has been used earlier in many studies<sup>8-14</sup> for the estimation of various inflammatory mediators levels throughout the studies to give precise measurements with least amount of human errors possible in manual estimation techniques.

A similar study was done by Lee KJ *et al*<sup>15</sup> in 2004 where the effect of light continuous forces and interrupted force on interleukin-1 and prostaglandin E2 was assessed *in vivo*. For the quadrant where continuous force was applied, there was significant upregulation of IL-1 beta after 24 hours of force application. The levels reduced thereafter but still remained higher than baseline levels. A second peak was seen after 28 days of force application which was due to the surge of cytokines due to their periodic recycling at around the same time period. However, on the interrupted side after initial peak at 24 hrs there was another peak after 1 week due to rhythmic activation of the appliance but there was an insignificant increase seen at second reactivation at second week which was postulated due to the feedback mechanism of cytokines over the inflammatory markers. The

**Table 1.** Group statistics to compare intragroup changes at the three time intervals using Students' unpaired t test

Time	Side	N	Mean	Std. Deviation	T
T1	R	20	298.85	22.354	1.612 p = 0.115
	L	20	287	24.092	
T2	R	20	346.65	27.683	1.58 p = 0.122
	L	20	330.95	34.759	
T3	R	20	325.05	27.927	2.59 p = 0.013
	L	20	349.75	32.195	

**Table 2.** Descriptive statistics to compare MPO values at the three time periods for right side (continuous force) and left side (intermittent force) using ANOVA

Side		N	Mean	Std. Deviation	F	p
R	T1	20	298.85	22.354	16.803	<0.001
	T2	20	346.65	27.683		
	T3	20	325.05	27.927		
	T1	20	287	24.092		
	T2	20	330.95	34.759		
	T3	20	349.75	32.195		

**Table 3.** Multiple comparison statistics used to compare value of MPO at a particular time period for the two sides with the value of MPO at the other two time periods using Bonferroni's t test

Side	(I) time	(J) time	Mean Difference (I-J)	P
R	T1	T2	47.8	<0.001
		T3	26.2	0.007
	T2	T3	21.6	0.034
L	T1	T2	43.95	<0.001
		T3	62.75	<0.001
	T2	T3	18.8	0.173

results of the study are concurrent with the findings of our study.

A study done by Grieve *et al*<sup>16</sup> in 1994 which measured IL-1 beta and PGE levels showed the values of these mediators significantly increased after 24 hours of force application but came back to baseline levels after 1 week of orthodontic tooth movement. Similarly, a study done by Uematsu *et al*<sup>6</sup> also showed increased levels of TNF- $\alpha$ , IL-1 and epidermal growth factor after 24 hours of appliance activation but dropped to normal levels after a week. The probable reason behind this was that force levels decay after a while leading to declining cytokine levels. Contrary to this study, a study done by Tzannetou *et al*<sup>17</sup> showed significant and constant increased levels of IL-1 beta till 28 days after a regular regime of rapid palatal expansion, which used heavy interrupted forces provided by screws. The possible reason given for this was the tendency of relapse of expansion bringing the two segments of maxilla together thereby increasing levels of inflammation or due to alterations brought about in the soft tissue.

The use of a split mouth design in the current study for the measurement of MPO between the two different nature of forces has proven to be a reliable and effective study design as demonstrated by an interesting study done by Yamaguchi M. *et al*<sup>11</sup> in 2009. He used conventional brackets in the maxillary left quadrant and the Damon system in the maxillary right quadrant. GCF samples were collected at specified time intervals. He concluded that the side on which self-ligating brackets were bonded showed significant lower levels of substance P, a potent marker of inflammation and pain, compared to the quadrant having conventional bracket system. On similar lines myeloperoxidase, also an inflammatory mediator, shows an initial high level of MPO suggesting increased degree of inflammation during course of orthodontic treatment in conventional bracket system.

In the present study, we have used NiTi closed coil springs to provide continuous forces but as has been shown by the study done by Cox C *et al*<sup>18</sup> in 2014, there is a statistically significant force decay which is approximately 12 percent after 4 weeks of activation and another 5 percent at 8 weeks. After this it remains steady. However, this decay was negated in our study since all the samples were collected within 3 weeks. Hence we

expected a reasonably constant amount of force throughout the study period.

Studies done by Kapoor P *et al*<sup>8</sup> and Ahmet Arif Celebi *et al*<sup>19</sup> demonstrated a significant correlation between gender and mediator levels. Mediators such as PGE2 and IL-1B have a significant correlation with the ovarian activity during orthodontic tooth movement. These mediator levels were found to be significantly and consistently lower in ovariectomized groups than in intact and ovariectomized groups whereas no such correlation has been established between myeloperoxidase and ovarian activity to our knowledge. This again shows the significance of measuring MPO levels without bothering about this confounding factor.

Multiple comparisons between MPO levels at a particular time point to the remaining timelines also revealed significant values. The only insignificant value found here was the change from T2 to T3 on the left side with intermittent force. The possible reason for this as mentioned by Richard J. Smith *et al*<sup>20</sup> in 1984 could be due to formation of wide area of hyalinization on the pressure side leading to formation of wide acellular zone and impediment of release of cytokines into the gingival crevice.

## CONCLUSION

The findings of this study show that the nature of the orthodontic force applied significantly affects inflammation, which was demonstrated by different levels of Myeloperoxidase with continuous and intermittent forces. Intermittent forces lead to significantly higher levels of inflammation compared to light, continuous forces and hence should be preferably avoided to bring about any kind of tooth movement.

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