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RESEARCH ARTICLE

EVALUATION OF MYELOPEROXIDASE AND LACTATE DEHYDROGENASE ENZYMATIC ACTIVITY IN GINGIVAL CREVICULAR FLUID IN ORTHODONTIC PATIENTS-AN INVIVO STUDY

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ABSTRACT

Aims and Objectives: To find out any association between Lactate dehydrogenase and Myeloperoxidase enzyme levels in the gingival crevicular fluid at various time interval during alignment of teeth in patients with different levels of crowding. **Materials and Method:** A total of 20 orthodontic patients were divided into 2 groups (10 patients in each groups) Group A (1-3 mm crowding) Group B (6-9 mm) based on the amount of crowding according to Little's irregularity index. The GCF sample is collected from the most affected tooth before activation of the appliance (T0), 2 hrs. After activation (T1), 7 days after activation (T2), 14 days after activation (T3) and at the end of alignment phase (T4) using micro capillary pipette. Enzyme quantification was done by ELISA method. **Results:** Independent sample t- test with unequal variance was done to compare the mean of MPO in minimal and maximum crowding. It was observed that, Maximum crowding displaying statistically significant higher Myeloperoxidase level at 2 hours of time interval compared to minimum crowding. (P =0.028). Similarly the mean of LDH in minimum and maximum crowding was compared. It was observed that, maximum crowding displaying statistically significant higher Myeloperoxidase level at 7th day (P=0.002), at 14th day (P=0.001) and after de-crowding (P=0.002) compared to minimum crowding. Maximum crowding displaying statistically significant higher Lactate dehydrogenase level at 2 hours of time interval compared to minimum crowding. (P =0.001). Contrarily, it was observed that, minimum crowding displaying statistically significant higher Lactate dehydrogenase level at 14 days of time interval compared to maximum crowding. (P =0.002).There is no statistically significant correlation found between Lactate dehydrogenase and Myeloperoxidase in minimum and maximum crowding cases at different time intervals. **Conclusion:** LDH and MPO activity can be measured with a quick method that is inexpensive and accessible to most laboratories and can be done on chairside with refinement.LDH and MPO activity can rapidly monitor possible deleterious effect of an excessive orthodontic force have been applied, and adjustments can be made according to individual response to orthodontic forces.

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INTRODUCTION

Tooth movement by orthodontic force application is characterized by remodelling 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 from physiological dental drift or tooth eruption. The orthodontic forces induce strain and 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 can evoke many cellular responses by various cell

types in and around teeth, providing a favourable microenvironment for tissue deposition or resorption.[1] Physiological tooth movement is a slow process that occurs mainly in the buccal direction into cancellousbone or because of growth into cortical bone. In contrast, orthodontic tooth movement can occur rapidly or slowly, depending on the physical characteristics of the applied force, and the size and biological response of the PDL.[2] To better describe the biological responses to orthodontic force in humans, non-invasive analyses of various cell mediators or enzymes in the gingival crevicular fluid (GCF) have also been performed. There are several inflammatory mediators in the GCF that we can use as a marker of tooth movement like IGF-1, 6, TNF-alpha, cytokines etc.[3] Inflammation is characterized by infiltration of leucocytes, among them, neutrophils (polymorphonuclear leukocytes). These polymorphonuclear cells have granules that contain myeloperoxidase (MPO); this enzyme produces oxidant molecules that can cause lipid peroxidation. The level of MPO activity is proportional to the number of polymorphonuclear cells in a tissue, reflecting the degree of inflammation.

MPO enzymatic activity analysis of saliva or gingival crevicular fluid (GCF) is a useful method for monitoring periodontal inflammation. This is particularly interesting because collection of the sample is not invasive, and the determination method is simple and accessible to standard laboratories.[2] Lactate dehydrogenase (LDH), an enzyme normally limited to the cytoplasm of cells, is only released extracellularly after cell death. Previous studies have demonstrated that the activity of LDH in GCF is significantly correlated with gingival inflammation and tissue destruction from periodontitis. Therefore, it has been proposed that LDH activity in the GCF is a potential marker for monitoring periodontal metabolism. With the consideration that LDH is an index of tissue destruction, and that, during orthodontic tooth movement, phenomena such as cell necrosis, have been described in the periodontal ligament, an increase in LDH activity in the GCF can be hypothesized.[4] Hence, MPO and LDH activity can be measured with a quick method that is inexpensive and accessible to most laboratories. MPO activity can rapidly monitor possible deleterious effects of an excessive orthodontic force has been applied, and adjustments can be made according to the individual response to orthodontic forces. The previous studies have assessed the activity of MPO and LDH enzymes till the 14th day after activation of appliance and limited literature is available regarding the MPO activity in GCF in the initial phase. Hence in this study we will be quantifying the enzymatic activity of MPO and LDH from the beginning of the treatment to the end of alignment phase with different levels of crowding. The aim of this present study is to find out the association between Lactate dehydrogenase and Myeloperoxidase enzyme levels in GCF at various time interval with different levels of crowding. The objectives include the assessment of the activity of myeloperoxidase and lactate dehydrogenase enzymes in patients with different levels of crowding at different time intervals of orthodontic treatment (0 hour, 2 hour, 7 days, 14 days & end of alignment) and at the same time to quantify the activity of myeloperoxidase and lactate dehydrogenase enzyme.

MATERIALS AND METHODOLOGY

The study comprised of 20 patients, including males and females with age between 12 and 30 undergoing orthodontic treatment selected from the department of orthodontics, DSCDS, Bangalore. The samples have been from patients who required orthodontic treatment and done the assessment, quantification and comparison of the levels of two inflammatory mediators, the study design is an experimental prospective study, with the power of 80% and alpha error of 5%.The Inclusion criteria are Patient requiring fixed orthodontic treatment with different levels of crowding, Patients between the age range of 12-30,Good general health, Healthy periodontal tissues with generalized probing depth of 3 mm or less, No use of anti-inflammatory drugs during and one month preceding the study.

20 orthodontic patients were divided into 2 groups Group A (minimum crowding 1-3 mm) and Group B(severe crowding 6-9 mm) with 10 patients in each groups based on the amount of crowding of teeth according to Little's irregularity index(Figure 1).Written informed consent was taken from all the patients. Patients were instructed to undergo complete scaling of the teeth before the collection of first GCF sample.The GCF sample is collected from the most affected tooth before activation of the appliance using micro capillary pipette (Figure 2). The collected samples were individually placed in buffer solution (50 mmol/L of Tris-hydrogen chloride, pH 7.4; 200 mmol/L of sodium chloride; 10 mmol/L of calcium chloride; and 0.02% of triton X-100-Anamol Laboratories). Enzyme quantification was done by ELISA method (Figure 3-Figure 6). The above mentioned steps were repeated at 2 hrs. After activation (T1), after 7 days of activation (T2), after 14 days of activation (T3) and at the end of levelling and aligning (T4).

RESULTS

Data analysis was done using SPSS version 20. Mean, standard deviation and percentage were used for the descriptive statistics. Twenty patients were enrolled for the study divided into two groups of mild and severe crowding with 10 patients in each group. In the present study, Myeloperoxidase and Lactate dehydrogenase enzyme activity were evaluated & tabulated in 20 subjects consisting of pretreatment (T0), at 2 hrs. After activation (T1), after 7 days of activation (T2), after 14 days of activation (T3) and at the end of levelling and aligning (T4).

Mean and standard deviation of lactate dehydrogenase among minimum and maximum crowding at different time intervals: According to Table 1, the mean value of LDH in the minimal crowding group at T0 is 97.51units/100 µL, T1 is 222.345units/100 µL, T2 is 185.19units/100 µL, T3 is 126.995units/100 µL and T4 is 116.48units/100 µL. While in the maximum crowding group, the LDH value at T0 is 97.57units/100 µL, T1 is 245.06units/100 µL, T2 is 184.76units/100 µL, T3 is 120.2units/100 µL and T4 is 115.57units/100 µL. Which is also shown in Graph 1. According to Table 2, the mean value of MPO in the minimal crowding group at T0 is 775.64units/100 µL, T1 is 2038.85units/100 µL, T2 is 942.52units/100 µL, T3 is 543.72units/100 µL and T4 is 871.13units/100 µL. While in the maximum crowding group at T0 is 792.72units/100 µL,

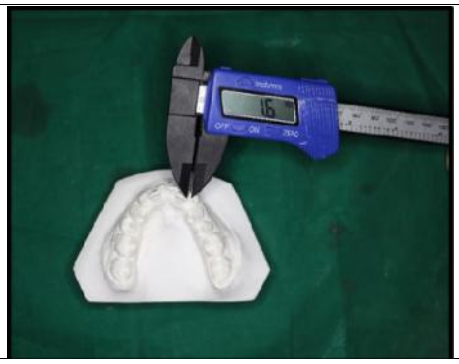


Figure 1: Measuring irregularity index

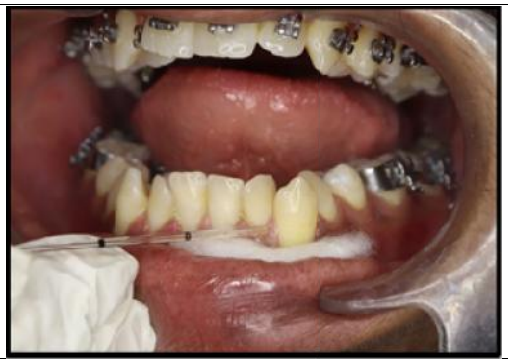


Figure 2: Collection of GCF sample

Figure 3: Incubation of samples after addition of buffer for MPO and LDH

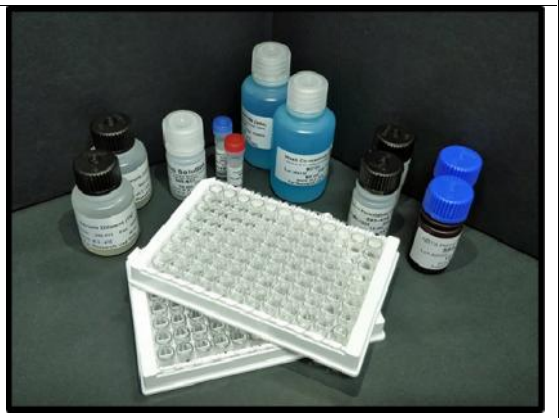


Figure 4: ELISA kit for MPO

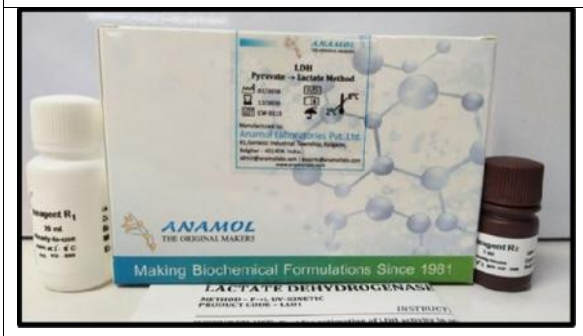


Figure 5: ELISA kit for LDH

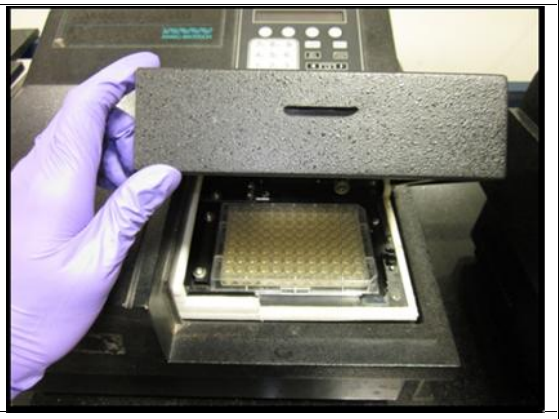


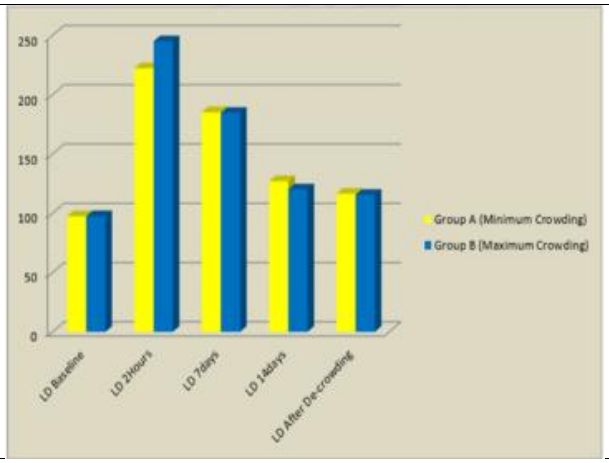
Figure 6: Optical density measurement for MPO and LDH

Table 1. Mean and standard deviation of Lactate dehydrogenase among minimum and maximum crowding at different time intervals

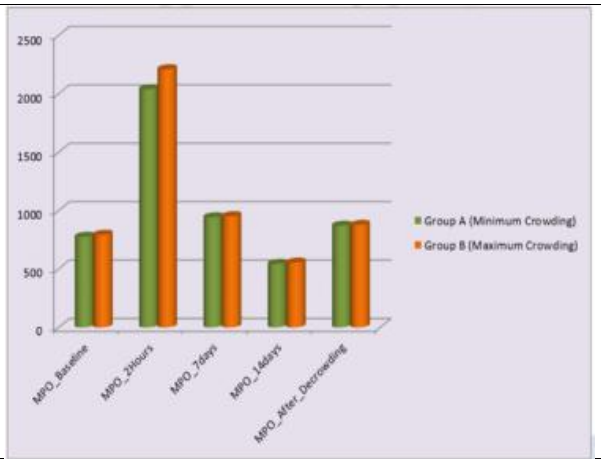
Groups		LDH Baseline	LDH 2Hours	LDH 7days	LDH 14days	LDH After Decrowding
Group A (Minimum Crowding)	Mean	97.51	222.345	185.19	126.995	116.48
	N	10	10	10	10	10
	Std. Deviation	0.67074	1.54532	1.7773	3.25068	1.29168
Group B (Maximum Crowding)	Mean	97.57	245.06	184.72	120.2	115.57
	N	10	10	10	10	10
	Std. Deviation	2.55258	5.82298	11.61137	4.86073	7.97553
Total	Mean	97.54	233.7025	184.955	123.5975	116.025
	N	20	20	20	20	20
	Std. Deviation	1.81671	12.36828	8.08816	5.32424	5.58022

Table 2: Mean and standard deviation of Myeloperoxidase among minimum and maximum crowding at different time intervals.

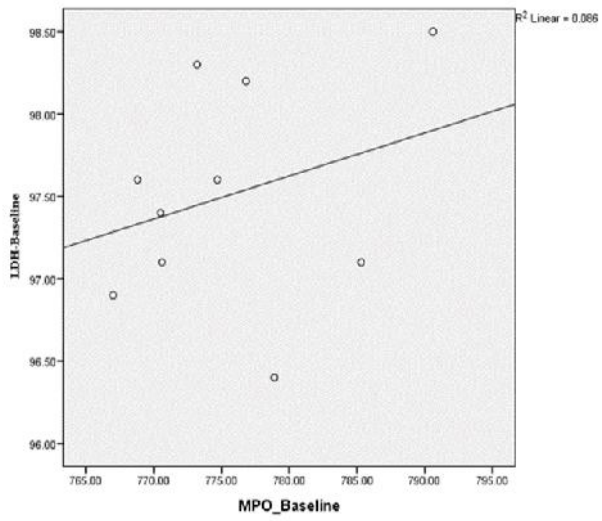
Groups		MPO Baseline	MPO 2Hours	MPO 7days	MPO 14days	MPO After De-crowding
Group A (Minimum Crowding)	Mean	775.64	2038.85	942.52	543.72	871.13
	N	10	10	10	10	10
	Std. Deviation	7.5217	8.83758	7.37335	7.76499	4.96433
Group B (Maximum Crowding)	Mean	792.72	2210.435	951.846	555.02	877.78
	N	10	10	10	10	10
	Std. Deviation	29.6096	206.9257	3.89128	4.44742	2.87549
Total	Mean	784.18	2124.643	947.183	549.37	874.455
	N	20	20	20	20	20
	Std. Deviation	22.77854	167.5323	7.47081	8.4577	5.21803



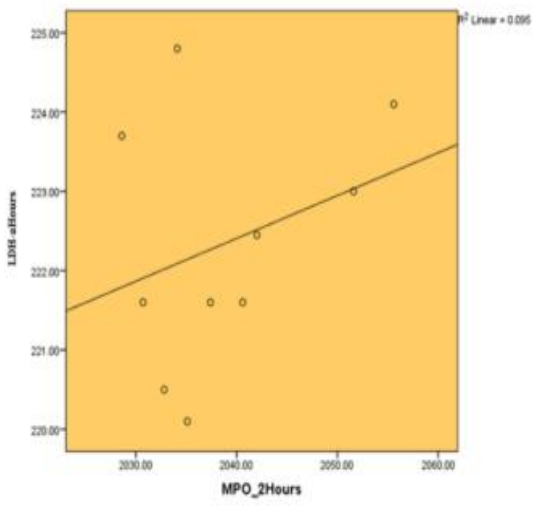
Graph 1: Mean and standard deviation of Lactate dehydrogenase among minimum and maximum crowding at different time intervals



Graph 2: Mean and standard deviation of Myeloperoxidase among minimum and maximum crowding at different time intervals



Graph 3: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at T0



Graph 4: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at T1

Table 3: Independent Samples Test shows Comparison of mean values of Myeloperoxidase between minimum crowding and maximum crowding subjects at different time intervals.

Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	Mean Difference	Sig. (2-tailed)	95% Confidence Interval of the Difference	
							Lower	Upper
MPO Baseline	Equal variances assumed	2.043	0.17	-1.768	-17.08	0.094	-37.3765	3.21652
	Equal variances not assumed			-1.768	-17.08	0.107	-38.5606	4.40059
MPO 2Hours	Equal variances assumed	195.428	**0.001**	-2.62	-171.585	**0.017***	-309.186	-33.9844
	Equal variances not assumed			-2.62	-171.585	**0.028***	-319.664	-23.5064
MPO 7days	Equal variances assumed	2.972	0.102	-3.537	-9.326	**0.002***	-14.865	-3.78704
	Equal variances not assumed			-3.537	-9.326	**0.003***	-14.9941	-3.65786
MPO 14days	Equal variances assumed	2.185	0.157	-3.993	-11.3	**0.001***	-17.2451	-5.35492
	Equal variances not assumed			-3.993	-11.3	**0.001***	-17.3561	-5.24393
MPO After De-crowding	Equal variances assumed	2.081	0.166	-3.666	-6.65	**0.002***	-10.4615	-2.83852
	Equal variances not assumed			-3.666	-6.65	**0.002***	-10.5303	-2.76974

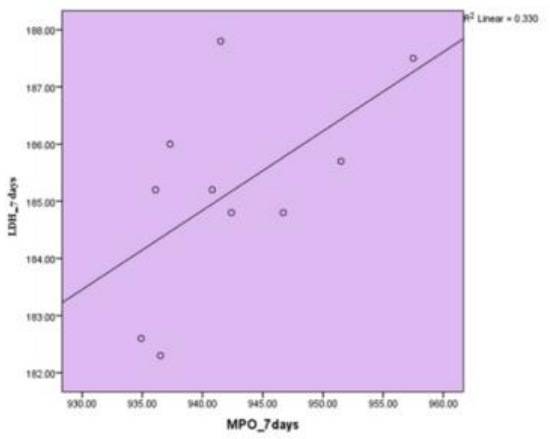
Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means				
		F	Sig.	t	Mean Difference	Sig. (2-tailed)	95% Confidence Interval of the Difference	
							Lower	Upper
LDH Baseline	Equal variances assumed	17.761	0.001	-0.072	-0.06	0.943	-1.81343	1.69343
	Equal variances not assumed			-0.072	-0.06	0.944	-1.91378	1.79378
LDH 2Hours	Equal variances assumed	2.683	0.119	-11.923	-22.715	0.001*	-26.7175	-18.7125
	Equal variances not assumed			-11.923	-22.715	0.001*	-26.9453	-18.4847
LDH 7days	Equal variances assumed	20.883	0	0.127	0.47	0.901	-7.33409	8.27409
	Equal variances not assumed			0.127	0.47	0.902	-7.87606	8.81606
LDH 14days	Equal variances assumed	0.217	0.647	3.675	6.795	0.002*	2.91008	10.67992
	Equal variances not assumed			3.675	6.795	0.002*	2.86906	10.72094
LDH After Decrowding	Equal variances assumed	19.125	0	0.356	0.91	0.726	-4.45774	6.27774
	Equal variances not assumed			0.356	0.91	0.73	-4.8261	6.6461

Table 5. Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at different time intervals.

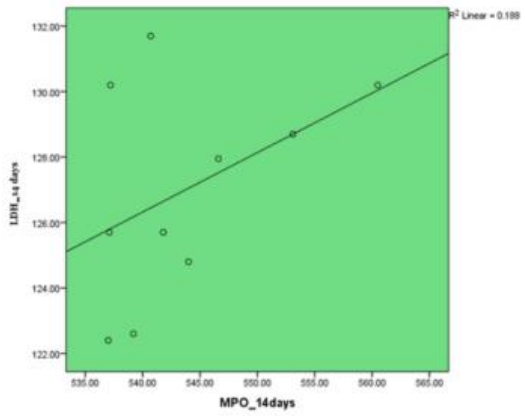
		MPO Baseline	MPO 2Hours	MPO 7days	MPO 14days	MPO After De-crowding
LDH Baseline	Pearson Correlation	0.293	0.242	0.256	0.281	0.224
	Sig. (2-tailed)	0.411	0.501	0.476	0.431	0.534
	N	10	10	10	10	10
LDH 2Hours	Pearson Correlation	0.392	0.309	0.554	0.448	0.533
	Sig. (2-tailed)	0.263	0.385	0.097	0.195	0.113
	N	10	10	10	10	10
LDH 7days	Pearson Correlation	0.434	0.347	0.575	0.479	0.545
	Sig. (2-tailed)	0.21	0.327	0.082	0.161	0.103
	N	10	10	10	10	10
LDH 14days	Pearson Correlation	0.371	0.294	0.547	0.434	0.532
	Sig. (2-tailed)	0.291	0.409	0.102	0.21	0.113
	N	10	10	10	10	10
LDH After De-crowding	Pearson Correlation	0.427	0.342	0.489	0.442	0.448
	Sig. (2-tailed)	0.218	0.333	0.152	0.201	0.194
	N	10	10	10	10	10

Table 6. Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among maximum crowding cases at different time intervals

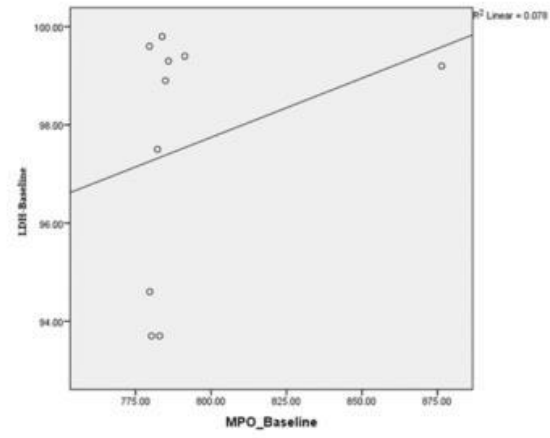
		MPO Baseline	MPO 2Hours	MPO 7days	MPO 14days	MPO After De-crowding
LDH Baseline	Pearson Correlation	0.28	0.632	0.449	0.191	0.533
	Sig. (2-tailed)	0.434	0.05	0.193	0.596	0.113
	N	10	10	10	10	10
LDH 2Hours	Pearson Correlation	-0.073	0.111	0.087	0.045	0.103
	Sig. (2-tailed)	0.841	0.761	0.811	0.902	0.778
	N	10	10	10	10	10
LDH 7days	Pearson Correlation	0.292	0.594	0.469	0.214	0.528
	Sig. (2-tailed)	0.413	0.07	0.172	0.553	0.117
	N	10	10	10	10	10
LDH 14days	Pearson Correlation	-0.105	0.207	0.002	-0.116	0.041
	Sig. (2-tailed)	0.772	0.567	0.996	0.75	0.911
	N	10	10	10	10	10
LDH After De-crowding	Pearson Correlation	0.24	0.491	0.38	0.164	0.475
	Sig. (2-tailed)	0.505	0.149	0.278	0.65	0.166
	N	10	10	10	10	10



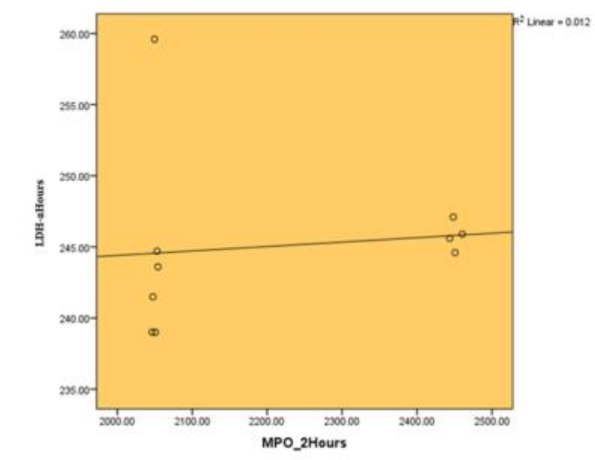
Graph 5: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at T2



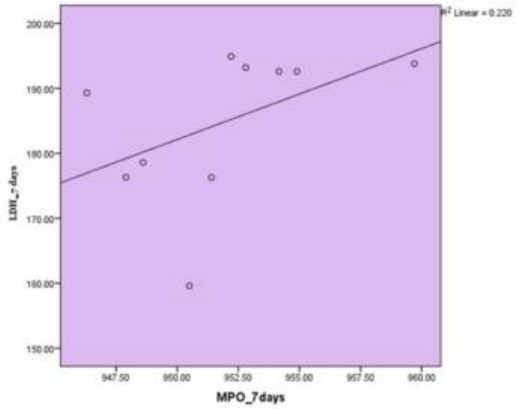
Graph 6: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at T3



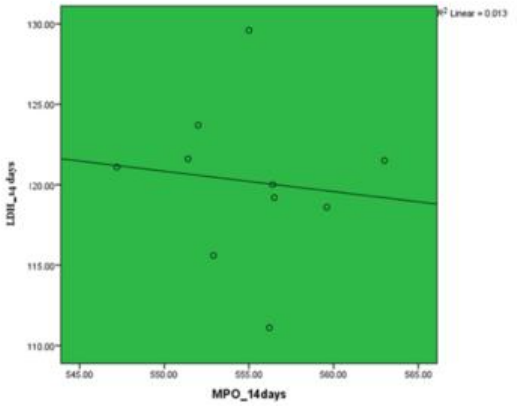
Graph 8: Pearson's correlation betweenLactate dehydrogenase and Myeloperoxidase among maximum crowding cases at T0



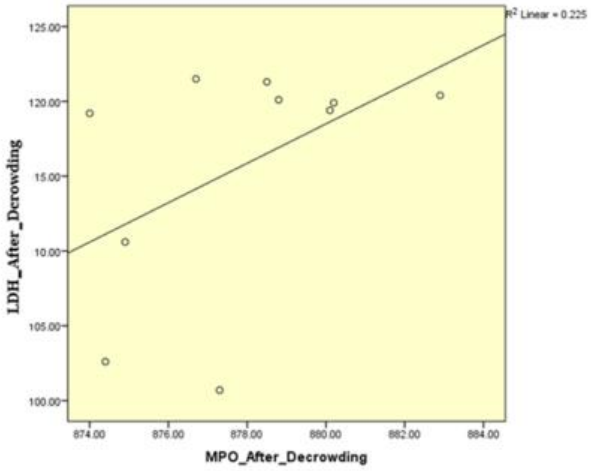
Graph 9: Pearson's correlation betweenLactate dehydrogenase and Myeloperoxidase among maximum crowding cases at T1



Graph 10: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among maximum crowding cases at T2



Graph 11: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among maximum crowding cases at T3



Graph 12: Pearson's correlation betweenLactate dehydrogenase and Myeloperoxidase among maximum crowding cases at T4

T1 is 2210.435units/100 µL, T2 is 951.846units/100 µL, T3 is 555.02units/100 µL and T4 is 877.78units/100 µL. Which is also shown in Graph 2.

Independent sample t test of myeloperoxidase: Independent Samples Test shows Comparison of mean values of Myeloperoxidase between minimum crowding and maximum crowding subjects at different time intervals. (Table 3) In the present study, value of MPO at T0 in minimum crowding group is 775.64 units/100 µL and maximum crowding group is 792.72 units/100 µL.MPO at T1 in minimum crowding group is 2038.85 units/100 µL and maximum crowding is 2210.435 units/100 µL.MPO at T2 in minimum crowding is 942.52 units/100 µL and maximum crowding is 951.846 units/100 µL.MPO at T3 in minimum crowding group is 543.72 units/100 µL and maximum crowding group is 555.02 units/100 µL.MPO at T4 in minimum and maximum crowding groups are 871.13 units/100 µL and 877.78 units/100 µL respectively. Levene's tests of equality of variance in different samples were used to determine the assumption of equal variances or not. Here t-test assumes the variance of the population from which different samples were drawn were equal. It tests the null hypothesis that the population variances were equal. The p value of Levene's Test for Equality of Variances here is 0.001 for MPO 2 hours, 0.102 for MPO 7 days, 0.157 for MPO 14 days and 0.166 for MPO after de crowding.

The p values of Levene's tests of equality were more than that of 0.05 except for MPO 2 hours. So the null hypothesis of equal variances was accepted for all except for MPO 2 hours, based on this, the P value of independent sample t-test with equal variance was assumed. By using, Independent sample t- test with unequal variance, it was observed that, Maximum crowding displaying statistically significant higher Myeloperoxidase level at 2 hours of time interval compared to minimum crowding. (P =0.028). Similarly, Independent sample t- test with equal variance, it was observed that, maximum crowding displaying statistically significant higher Myeloperoxidase level at 7th day (P=0.002) , at 14th day (P=0.001) and after de-crowding (P=0.002) compared to minimum crowding.

Independent sample t test of lactate dehydrogenase: Independent Samples Test shows Comparison of mean values of Lactate dehydrogenase between minimum crowding and maximum crowding subjects at different time intervals. (Table 4). In the present study, LDH value at T0 in minimum crowding group is 97.51units/100 µL and maximum crowding group is 97.57 units/100 µL. LDH at T1 in minimum crowding group is 222.345 units/100 µL and maximum crowding group is 245.06 units/100 µL. LDH at T2 in minimum crowding group is 185.19units/100 µL and maximum crowding group is 184.72 units/100 µL. LDH at

T3 in minimum crowding group is 126.995 units/100 µL and maximum crowding group is 120.2 units/100 µL. LDH value at T4 in minimum and maximum crowding are 116.48 units/100 µL and 115.57 units/100 µL respectively. Levene's tests of equality of variance in different samples were used to determine the assumption of equal variances or not. Here t-test assumes the variance of the population from which different samples were drawn were equal. It tests the null hypothesis that the population variances were equal. The p value of Levene's Test for Equality of Variances here is 0.119 for LDH 2 hours and 0.647 for LDH 14 days. The p values of Levene's tests of equality were more than that of 0.05. So the null hypothesis of equal variances was accepted, based on this, the P value of independent sample t- test with equal variance was assumed. By using, Independent sample t- test with equal variance, it was observed that, Maximum crowding displaying statistically significant higher Lactate dehydrogenase level at 2 hours of time interval compared to minimum crowding. (P =0.001). Contrarily, it was observed that, minimum crowding displaying statistically significant higher Lactate dehydrogenase level at 14 days of time interval compared to maximum crowding. (P =0.002).

Correlation between lactate dehydrogenase and myeloperoxidase among minimum crowding cases: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among minimum crowding cases at different time intervals (Table 5). According to Table 5, the correlation significance of MPO and LDH at T0, T1, T2, T3 and T4 are found to be 0.411, 0.385, 0.082, 0.21 and 0.194 respectively. Pearson's correlation shows there is no statistically significant correlation found between Lactate dehydrogenase and Myeloperoxidase in minimum crowding cases at different time intervals. The correlation between MPO and LDH at T0, T1, T2, T3 and T4 are shown in Graph3, Graph 4, Graph 5, Graph 6, and Graph 7 respectively.

Correlation between lactate dehydrogenase and myeloperoxidase among maximum crowding cases: Pearson's correlation between Lactate dehydrogenase and Myeloperoxidase among maximum crowding cases at different time intervals (Table 6). According to Table 6, the correlation significance of MPO and LDH at T0, T1, T2, T3 and T4 are found to be 0.434, 0.761, 0.172, 0.75 and 0.166 respectively. Pearson's correlation shows there is no statistically significant correlation found between Lactate dehydrogenase and Myeloperoxidase in maximum crowding cases at different time intervals. The correlation of MPO and LDH among maximum crowding cases at T0, T1, T2, T3 and T4 are shown in Graph 8, Graph 9, Graph 10, Graph 11, and Graph 12 respectively.

DISCUSSION

Drugs are the medicines used to cure diseases whereas force is the medicine used to cure malocclusions in orthodontics. Orthodontic force has been defined as "force applied to teeth for the purpose of effecting tooth movement, generally having a magnitude lower than an orthopaedic force,".[5]The classic definition of optimal force by Schwarz in 1932 was "the force leading to a change in tissue pressure that approximated the capillary vessels' blood pressure, thus preventing their occlusion in the compressed periodontal ligament."[6]

Traditionally, orthodontic forces have been categorized as "light" or "heavy," and it was assumed that light forces are gentler and therefore more physiologic than heavy forces. Unlike light forces, heavy forces often cause necrosis (hyalinization) of the PDL and undermining bone resorption, and have been implicated in root resorption.[7] The early phase of orthodontic tooth movement involves an acute inflammatory response, characterized by periodontal vasodilatation and migration of leucocytes out of the capillaries followed by a chronic process that is mainly proliferative, involving fibroblasts, endothelial cells, osteoblasts, and alveolar bone marrow cells.[8]This concurrent phase of acute and chronic changes results in the osteoclastic bone resorption and osteoblastic bone deposition which is called as the remodelling process. Remodelling changes in the alveolar bone and the PDL induce production of various cell mediators or enzymes that can be used as biomarkers of orthodontictreatment.[9, 10]The early works by Last et al, [11] and Embery and Waddington[9] described many glycosaminoglycan's, proteoglycan and tissue proteins in GCF, providing evidence for the presence of underlying state of biochemical reflections in paradental tissues. A reflection of these phenomena can be found in the saliva and gingival crevicular fluid around the moving teeth, where significant elevations in the concentrations of inflammatory mediators, such as cytokines and prostaglandins are seen.[12] Several studies have focused on the composition of saliva and the changes that occur during orthodontic tooth movement[13,14], but very few studies have concentrated on the gingival crevicular fluid changes during orthodontic tooth movement. The mechanism of bone remodelling during orthodontic treatment is related, on one hand to the release of inflammatory mediators, such as prostaglandin-E2 and interleukin 1-beta,[13] and on the other hand to the production of neuropeptides, such as substance P, Interleukin-1 β, a known potent cytokine produced mainly by activated monocytes, participates in the initiation of bone resorption[15,16]either by activating osteoclasts or by stimulating the synthesis of prostaglandin-E2.[15] It is well known that when a force is applied to a tooth, the periodontal tissues undergo either tension or compression stress, depending on the tooth movement.[17,18]

During orthodontic tooth movement, the early response of periodontal tissues to mechanical stress is an acute inflammatory reaction characterized by infiltration of neutrophils which have granules that contain MPO. The level of MPO activity is proportional to the number of polymorphonuclear cells in a tissue, reflecting the degree of inflammation. [(19,20)] In the current study, it was observed that, Maximum crowding displaying statistically significant higher Myeloperoxidase level at 2 hours of time interval compared to minimum crowding. (P =0.028). Similarly, Independent sample t- test with equal variance, it was observed that, maximum crowding displaying statistically significant higher Myeloperoxidase level at 7th day (P=0.002) , at 14th day (P=0.001) and after de-crowding (P=0.002) compared to minimum crowding. These findings are consistent with those of Navarro-Palacios *et al*.[21] showed that the MPO activity in the saliva remained elevated at 2 h and day 7, but MPO activity in the GCF increased at 2 h; by day 7, a diminution was observed. This indicated that GCF can be a more confirmatory medium that accurately reflects inflammatory changes than saliva. GCF is produced directly in the gingival sulcus and by extravasation of circulating

plasma. Saliva, in contrast, is produced by the salivary glands. Although saliva contains substances similar to GCF, it reflects the buccal environment more than the tooth environment. Therefore, GCF likely reflects local tooth inflammation caused by orthodontic movement more accurately than saliva. This could explain the different patterns of MPO activity that the author observed between GCF and saliva. In contrast to the above results, investigation done by Marcaccini*et al*.[22] with respect to GCF whose study showed that MPO activity is highly increased 2 h after appliance activation in both GCF and saliva, and that it decreases to baseline levels after 7 days. In their study, there was no statistically significant difference between MPO levels collected at 7 and 14 days although a lower value was observed on day 14 in both saliva and GCF. The present study, however, did not aim to assess the MPO levels in the saliva. The study done by Honey Gurbaxani et al [23] to assess myeloperoxidase (MPO) activity at different force levels in gingival crevicular fluid (GCF) during the initial phase of orthodontic tooth movement by varying the effective force levels also found that There was a highly significant increase in the MPO levels in the GCF at 14th day after force application which can be correlated to the onset of inflammatory reactions in the periodontium. Lactate Dehydrogenase, an enzyme normally limited to the cytoplasm of cells, is only released extracellularly after cell death. Previous studies have demonstrated that the activity of lactate dehydrogenase in gingival crevicular fluid is significantly correlated with gingival inflammation [24] and tissue destruction from periodontitis. [25,26]Till date no studies have been done evaluate the LDH level in different time intervals of initial orthodontic treatment. In the present study, it was observed that, Maximum crowding displaying statistically significant higher Lactate dehydrogenase level at 2 hours of time interval compared to minimum crowding. (P =0.001). Contrarily, it was observed that, minimum crowding displaying statistically significant higher Lactate dehydrogenase level at 14 days of time interval compared to maximum crowding. (P =0.002).

The study done by Emanuela Serra et al, [3] to examine the lactate dehydrogenase (LDH) activity in GCF to assess whether GCF LDH can be proposed as a sensitive marker for periodontal tissue modifications during orthodontic tooth movement, initially indicated a possible role of GCF LDH during the early phases of orthodontic treatment and therefore warrant further study as a possible diagnostic tool for tissue response during orthodontic treatment. In thispresent study we compared and analysed the concurrent proportional changes of LDH and MPO in GCF to find the variation of both the enzymes at different time intervals in patients with different levels of crowding. Our results shows that the proportional changes of LDH in GCF is more or less the same as that of MPO in GCF, but not statistically significant. Due to the difficulty in GCF sampling and analysis procedures, heterogeneity of results are common in GCF studies. Thus, it is suggested that the future studies should focus on refinement of GCF sampling to yield concrete results which can be done on chair side. Hence, MPO and LDH activity can be measured with a quick method that is inexpensive and accessible to most laboratories. Considering the basic fact that neutrophils form the first line of defense mechanism for inflammation following application of orthodontic tooth movement, MPO exhibited by the neutrophils' granules would be the first to be exhibited in the GCF. Collecting GCF samples is not invasive; then, MPO and LDH activity can rapidly monitor possible deleterious effects of an excessive orthodontic force has been applied, and adjustments can be made according to the individual response to orthodontic forces. Further studies, targeting large sample size and different force levels with different appliances in different stages of treatment is required for a better insight and understanding of the role of myeloperoxidase and lactate dehydrogenase during orthodontic tooth movement.

Conclusion

- A study was done to find out the correlation between myeloperoxidase and lactate dehydrogenase in gingival crevicular fluid of patients with different levels of crowding in different time intervals.

The conclusion of the study is as follows:

- The nature of the orthodontic force applied significantly affects inflammation, which was demonstrated by different levels of Myeloperoxidase and Lactate dehydrogenase enzymes in minimum and severe crowding groups.
- It was observed that, Maximum crowding displaying statistically significant higher Myeloperoxidase level at 2 hours of time interval compared to minimum crowding. Similarly, it was observed that, maximum crowding displaying statistically significant higher Myeloperoxidase level at 7th day, at 14th day and after de-crowding compared to minimum crowding.
- It was observed that, Maximum crowding displaying statistically significant higher Lactate dehydrogenase level at 2 hours of time interval compared to minimum crowding. Contrarily, it was observed that, minimum crowding displaying statistically significant higher Lactate dehydrogenase level at 14 days of time interval compared to maximum crowding.
- There is no statistically significant correlation found between Lactate dehydrogenase and Myeloperoxidase in minimum and maximum crowding cases at different time intervals.
- LDH and MPO activity can be measured with a quick method that is inexpensive and accessible to most laboratories and can be done on chairside with refinement.LDH and MPO activity can rapidly monitor possible deleterious effect of an excessive orthodontic force have been applied, and adjustments can be made according to individual response to orthodontic forces.

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