



The Effect of Obesity Versus Orthodontic Tooth Movement on Salivary Myeloperoxidase Level

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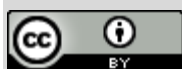
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Abstract

Background: Obesity is a major healthcare challenge and orthodontic treatment need is steadily increased thus it is necessary to understand the response of teeth and their supporting structures of obese subjects, to the applied orthodontic force, by noninvasive analysis of inflammatory biomarkers in saliva. **Aim of current study:** This prospective biochemical study investigated the levels of the inflammatory mediator myeloperoxidase (MPO) during orthodontic tooth movement in obese and normal-weight individuals undergoing routine orthodontic treatment with fixed appliances. **Patients and methods:** Sixty patients with mean age 14.5 (SD, 1.8) years and body mass index (BMI) mean of 30.75 (2.46) kg/m² in obese and 19.48 (2.12) kg/m² in normal weight groups were followed before fixed appliance placement till 60 days. Data collection included unstimulated mouth saliva (UMS) sample at: (T0) baseline (before appliance placement); (T1) 1-day and (T2) 7-days following appliance placement; (T3) 60-days. MPO levels were measured in UMS between T0-T3.

Results were analyzed by Friedman and Mann Whitney U test. **Results:** The level of MPO was significantly greater in UMS of obese patients at baseline (T0) and all subsequent time-points (T1-T3). Within each group, MPO level increased from T0 till T3 with no significant difference between T2-T3 in obese group and between T0-T1, and T2-T3 in control group.

Conclusion: The level of MPO was significantly greater in UMS of obese patients at baseline, consistent with a systemic pro-inflammatory state of obesity. Orthodontic treatment induced increased MPO levels in UMS within both groups. MPO levels can be reliably determined in saliva.

Introduction:

Obesity can be described as a subclinical chronic inflammation characterized by production of pro-inflammatory mediators by excess adipose tissue⁽¹⁾. Obesity was steadily increased amongst children and adults for the last few decades representing a serious healthcare issue due to the strong correlations between elevated body mass index (BMI) and a number of chronic illnesses, which culminate in an overall increased risk of mortality⁽²⁻³⁾. In relation to oral health, there is evidence of periodontal diseases in obese subjects were affected by different inflammatory and metabolic markers in comparison to normal weight⁽⁴⁾. Also studies showed a clear relation between fixed orthodontic appliance⁽⁵⁾ and obesity with periodontal health both clinically and radiographically⁽⁶⁾ and change in salivary constituents even with good oral health⁽⁷⁾.

Orthodontic tooth movement (OTM) relies upon successive responses to external mechanical force in surrounding periodontal tissue and alveolar bone, interposed by an acute inflammation in which there is a dilatation of blood vessels and recruitment, differentiation and proliferation of appropriate inflammatory cells⁽⁸⁾. Amongst these cell populations, polymorphonuclear neutrophils (PMN) play a key role which liberated from the granules inside the cells⁽⁹⁾ containing the myeloperoxidase (MPO) enzyme, which is a host derived compound essential for the protection that confirmed by PMNs⁽¹⁰⁾. It is established that a useful method to monitor the periodontal inflammation is through the enzymatic activity of MPO in gingival crevicular fluid and/or saliva⁽¹¹⁻¹³⁾, with levels of MPO activity that correlated with the number of PMNs in tissues⁽¹¹⁾. Thus, MPO levels found in gingival crevicular fluid during OTM can represent the extent of PMN infiltration and provide a precious technique in evaluating the level of inflammation resulting from the applied orthodontic force. Indeed, there is evidence of increased MPO activity in gingival crevicular fluid and saliva two hours following the application of orthodontic force, in comparison to their baseline

levels⁽¹³⁾, although this is not associated with any significant differences between individuals with minimal or severe crowding⁽¹⁴⁾. This prospective study has tested the impact of OTM and/or obesity on salivary MPO levels both prior to and through the process of orthodontic alignment in patients undertaking orthodontic therapy using fixed orthodontic appliances.

Patients and method: This prospective study was approved by the Research Ethical Committee of Ashur University, College of Dentistry (Ref. No. 1073), comparing the influence of obesity on salivary MPO levels during the alignment stage of orthodontic treatment using fixed appliances for participants attending multiple private clinics in different places of Baghdad city from December 2023 till January 2025.

Eligibility criteria included: (1) patients receiving orthodontic treatment using fixed appliance; (2) age range of 12-18 years; (3) without any medical issues or medication (antibiotic therapy in the last six months); (4) All participants were non-smokers; (5) permanent teeth. Height (in centimeter) and body weight (in kilogram) were measured by the same operator (researcher). BMI was calculated as weight (kg) divided by height in meters squared (kg/m^2). BMI centile in relation to age and sex was calculated according to the growth chart of Royal College of Paediatrics and Child Health⁽¹⁵⁾.

The data was collected at (T0) before the insertion of orthodontic appliance (baseline); (T1) 1-day after the insertion of orthodontic appliance; (T2) 7-days after the insertion of orthodontic appliance; and (T3) at 60-days following the insertion of orthodontic appliance.

From each patient, unstimulated mouth saliva (UMS) was collected for 5 minutes by drooling method using a pre-weighed sterile tube, and then it was centrifuged at 10000 rpm. The processed UMS was allocated in Eppendorf Tubes (500 μl), labeled, then stored in -20 °C freezers. Sample was analysed by Elissa assay using a specific kit (R&D systems,

Abingdon, UK) for detection (in pg/mL) of the inflammatory mediator MPO.

Statistical methods: The outcomes of this study were summarized using descriptive statistics. Shapiro-Wilk test for normality was used to check the data distribution before analysis was performed. To assess the variation in the level of non-normally distributed salivary MPO levels during study time, the Friedman test was used for both the control (normal weight) and obese groups, followed by a paired test.

The non-normally distributed MPO concentration values at each time point were compared between the normal weight and obese groups using the Mann Whitney U test. In this study SPSS (BM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 23, Armonk, New York, IBM Corp) was used for analysing data and the significance level was set at $p \leq 0.05$.

Results:

A total of sixty patients, 30 obese with the age mean of 14.3 (SD=1.78) and 30 control (normal weight) with the age mean of 14.7 (SD=1.87) years, and mean BMI of 30.75 kg/m² (SD=2.46) in obese and 19.48 kg/m² (SD=2.12) in control (normal weight) groups (Table 1) were followed from baseline (before fixed appliance insertion) till 60 days. The UMS was collected from those patients before appliance insertion (T0), 1-day following insertion of fixed appliance (T1), 7-days following insertion of fixed appliance (T2) and 60-days after the insertion of fixed appliance (T3).

Normality test showed that the age and BMI of patients were normally distributed whereas; MPO concentration data was not normally distributed. T-test showed significant difference ($p < 0.001$) in the BMI between obese and control (normal weight) patients as shown in Table (1).

Biochemical analyses, Mann Whitney U test revealed that salivary MPO levels were significantly greater in obese patients in comparison to control (normal weight) patients at all-time points (T0 –T3) as shown in Table (2).

Friedman test showed that in both control and obese groups the salivary MPO level

was significantly increased from the baseline (before appliance placement) T0 with a further increase at day 1, day 7 and day 60 following appliance insertion (T1-T3) as shown in Table (3) and Figure (1).

Pairwise test for multiple comparison showed that in obese group a significant increase was found between T0, T1, and T2, however; this increase was not significant between T2 and T3. In control (normal weight) group the increase was not significant between T0-T1 and T2-T3 but it was significant between others as shown in Table (4).

Discussion

Orthodontic treatment with fixed-appliances produces many changes in the periodontium, particularly the induction of inflammation. This prospective biochemical investigation conducted for a group of control (normal weight) and obese to demonstrate the effect of body weight variation on the inflammatory reaction during the alignment (first 2 months) of orthodontic treatment with fixed appliance. A key event in inflammation is the recruitment of PMNs which liberate the MPO enzyme from their granules⁽⁹⁾. Since MPO levels represent the infiltration of PMN; therefore determining MPO levels might assess the degree of inflammation.

Inflammatory changes during OTM leads to the release of multiple inflammatory mediators, which often can be detected in gingival crevicular fluid⁽¹⁶⁾. The rationale for using saliva is that the composition of whole saliva is quite similar to that of gingival crevicular fluid⁽¹⁷⁻¹⁸⁾, saliva can be collected non-invasively, determined simply and laboratory investigations are available⁽¹³⁾. Therefore, in the present study UMS was analysed. The groups of this study were equivalent at the baseline statistics, with only significant difference in BMI.

The results revealed that MPO levels in UMS was greater in obese at the baseline due to greater number of periodontal tissue neutrophil of obese subjects as a result of pro-inflammatory state of obesity leading to greater levels of MPO in UMS even before beginning the orthodontic treatment

which in line with previous studies reported the association of MPO with obesity⁽¹⁹⁻²²⁾. The increased MPO levels was also previously observed in association with fixed appliance orthodontic treatment⁽¹³⁾; however, the results of this study revealed that the effect of both OTM and obesity on the levels of salivary MPO that can not be compared clearly with previous studies used other samples such as gingival crevicular fluid or serum. On the other hand, during orthodontic treatment, teeth were probably subjected to different range of force that can affect the impact of force on the number of PMNs present in the gingival tissues which in turn affects on MPO level. However, in the present study the effect of various malocclusion and orthodontic forces were minimized based on previous study found comparable levels of MPO in saliva and gingival crevicular fluid irrespective to the amount of dental crowding⁽¹⁴⁾.

Interestingly, there was also some evidence of increased circulating MPO levels in obese individuals compared to control (normal weight), which show high level even following weight loss and bariatric surgery⁽¹⁹⁾. Similarly, increased levels of systemic MPO have been found in obese individuals when compared to control (normal weight)⁽²¹⁻²²⁾, and obese individuals with potential cardiovascular risk with or even without inflammation⁽²⁰⁾. The strengths of this study comes from its design which is prospective⁽⁴⁾, experimental groups are comparable before the onset of the study, and at the

end of the study there was no drop out and obesity was assessed according to a reliable measure. However, to confirm the systemic change of MPO level with OTM and/or obesity needs to assess MPO level in serum sample of these control (normal weight) and obese groups, which is considered invasive and has an ethical issue. Furthermore, the patients of this study were selected from many different clinics thus it was difficult to arrange sample collection at only morning or afternoon day time.

Conclusion:

This biochemical study showed that MPO concentration was higher in saliva of obese patients at baseline consistent with a systemic pro-inflammatory state of obesity. Orthodontic treatment induced increased MPO levels in saliva in both groups. Therefore MPO levels during orthodontic alignment can be reliably determined in saliva samples.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study

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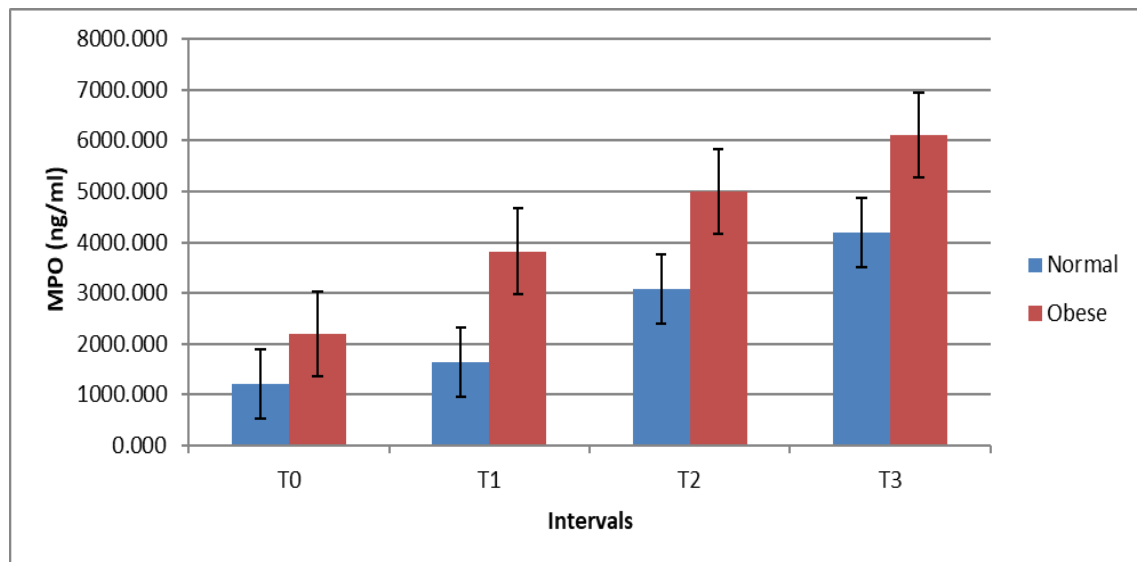


Figure (1): MPO concentration (ng/ml) in USM of normal weight and obese groups.

Table (1): Body Mass Index (BMI) in normal weight and obese groups

Groups	N	Mean	S.D.	Min.	Max.	Mean difference	t-test	p-value
Normal	30	19.484	2.121	16	24.5	-11.269	-18.98	0.001
Obese	30	30.753	2.465	28.8	37.4			

Table (2); Descriptive statistics and Mann Whitney U test comparison for normal weight and obese groups.

Parameters	Groups	Descriptive statistics					Comparison	
		N	Median	IQR	Mean Rank	Sum of Ranks	MWU test	p-value
T0	Normal	30	1214.230	1074.55-1455.57	25.67	770	305	0.023
	Obese	30	2188.205	946.908-2876.410	35.33	1060		
T1	Normal	30	1633.590	941.75-3381.31	18.87	566	101	0.001
	Obese	30	3819.255	2719.660-484.383	42.13	1264		
T2	Normal	30	3087.460	2771.39-3381.31	16.53	496	31	0.001
	Obese	30	5000.765	4390.380-6858.570	44.47	1334		
T3	Normal	30	4192.240	3416.07-5063.61	24.27	728	236	0.006
	Obese	30	6106.435	4445.888-7050.393	36.73	1102		

Table (3): Comparison of the MPO levels among different time points (T0-T3) in normal weight and obese groups using Friedman test.

Groups	Parameters	Descriptive statistics				Comparison By Friedman Test	
		N	Median	IQR	Mean Rank	X ² test	p-value
Normal	T0	30	1214.23	1074.55-1455.57	1.40	70.2	0.001
	T1	30	1633.59	941.75-3381.31	1.70		
	T2	30	3087.46	2771.39-3381.31	3.10		
	T3	30	4192.24	3416.07-5063.61	3.80		
Obese	T0	30	2188.21	946.908-2876.410	1.17	53.88	0.001
	T1	30	3819.26	2719.660-484.383	2.30		
	T2	30	5000.77	4390.380-6858.570	3.27		
	T3	30	6106.43	4445.888-7050.393	3.27		

Table (4): Pairwise test after Friedman test for multiple comparison.

Groups	Normal	Obese
T0-T1	1	0.004
T0-T2	0.001	0.001
T0-T3	0.001	0.001
T1-T2	0.001	0.022
T1-T3	0.001	0.022
T2-T3	0.214	1

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