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Assessment of salivary tumor necrosis factor-alpha level in the initial stages of treatment with fixed appliances and clear aligners

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Abstract

OBJECTIVES: To assess and compare the tumor necrosis factor-alpha (TNF-α) levels in saliva samples during the initial stages of orthodontic treatment with fixed orthodontic appliances (FAs) and clear aligners (CAs).

MATERIALS AND METHODS: This longitudinal study comprised 40 patients (22 males, 18 females, mean age 22 ± 7 years) who were categorized into two equal-sized groups. Group A comprised 20 patients treated with FA, and Group B comprised 20 patients treated with CA. Unstimulated saliva was collected before the intiation of treatment and then collected again after the placement of the FA/CA at 24 hrs, 7th day, and on the 21st day in both groups. TNF-α levels were determined through ELISA.

STATISTICAL ANALYSIS: The data were subjected to statistical analysis. For intragroup comparison of TNF-α at different time points, the Wilcoxon matched-pairs signed-rank test was used, and for intergroup comparison of FAs and CAs at different time points, the Mann–Whitney U test was used.

RESULTS: TNF-α levels in the saliva increased significantly at 24 hours, followed by a decline on the 7th day and 21st day in both groups. Changes in TNF-α levels were significantly higher in the FA group than those in the CA group at different time points.

CONCLUSION: This study showed that the salivary TNF-α levels increased significantly during the initial stages of FA and CA treatment at different time points. The mean salivary TNF-α level in both FA and CA groups increased significantly at 24 hours, followed by a decline on the 7th day and then on the 21st day. There was a significant difference between the FA and CA treatment, where the CAs showed a significantly low level of TNF-α in saliva at different intervals of time when compared to the FAs.

Keywords:

Clear aligner, fixed appliance, saliva, tumor necrosis factor-alpha

Introduction

During orthodontic treatment, external stimuli are exerted on the teeth, causing an inflammatory response in the periodontal tissue. Biological processes associated with alveolar bone resorption and apposition are triggered by these inflammatory mediators.[1]

One such cytokine, tumor necrosis factor-alpha (TNF-α), is of particular importance in orthodontic tooth movement as it is an early modulator of bone resorption[2-5] and is detectable in the gingival sulcus.[6] TNF-α, also known as cachectin, is a polypeptide cytokine produced by macrophages and monocytes. TNF-α functions as a potent pyrogen and further acts as a multipotent modulator of immune response.[7,8] Several studies

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have attempted to determine the levels of cytokines during orthodontic tooth movement.[9,10] Lowney *et al*.[11] reported elevation of TNF-α because of orthodontic force. This cytokine is produced primarily by macrophages and activated monocytes and also by osteoblasts and is an activator of osteoclastic bone resorption.[12,13]

The use of saliva is a simpler and non-invasive method than the assessment of serum and urine in the dental office. Studies have shown that saliva could be used as fluid for the assay of human biomarkers of bone turnover.[14]

Nowadays, clear aligners (CAs) are in demand as a comfortable and aesthetic alternative for fixed orthodontic appliances (FAs).[15] CAs work like any other orthodontic appliances. They apply pressure, which gradually moves the teeth and remodels the supporting bone.[16] Studies assessing the treatment efficiency of CAs at the tissue and cellular level are limited. Thus, this study aimed to identify and compare the salivary TNF-α levels in the CAs and FAs during the initial stages of orthodontic treatment.

Materials and Methods

Subjects

In this longitudinal study, 40 patients (22 males, 18 females, mean age 22 ± 7 years) requiring orthodontic treatment were consulted and selected. Patients having mild to moderate crowding (1–6 mm of crowding) according to Little's irregularity index were included in this study.[17] Patients were categorized into two groups: Group A (20 patients treated with FAs) and Group B (20 patients treated with CAs). Ethical clearance was obtained from the Institutional Review Board and Ethical Committee. Informed consent was obtained from patients and parents/guardians.

Inclusion and exclusion criteria

Patients with mild to moderate crowding according to Little's irregularity index, healthy periodontal tissue, probing depth values ≤3 mm in the whole dentition, no history of surgery in the oral cavity, no history of systemic diseases, no history of any childhood debilitating diseases, and no use of anti-inflammatory drugs, steroids, or antibiotics during the past 3 months were included in the study. Patients with poor periodontal status or systemic diseases and those on any other medications were excluded from the study.

Clinical procedure

Unstimulated saliva was collected using the draining method by asking the patients to swallow first and then tilt their head forward and expectorate all saliva into a plastic cup within 10 minutes.[18] The first saliva sample was collected before the placement of the appliance (T0), and subsequent samples were collected after the placement of the appliance at 24 h (T1) and on the 7th day (T2) and 21st day (T3).

Preparation of collected salivary samples

Saliva was centrifuged at 3000 rpm for 10 minutes to separate cells and large macromolecules. The supernatant was collected and used for analysis of the biomarker. The collected samples were stored at -20 till analysis.

TNF-α assay

TNF-α levels in saliva samples were evaluated in both FA and CA groups as TNF-α is a salivary biomarker of bone turnover. The concentration of TNF-α was determined using the enzyme-linked immunosorbent assay (Weldon Biotech, India).

Statistical analysis

The collected data were statistically analyzed using SPSS (version 20.0 Armonk, NY: IBM Corp). For intragroup comparison of TNF-α levels at different time points, the Wilcoxon matched-pairs signed-rank test was used, and for intergroup comparison of FAs and CAs at different time points, the Mann–Whitney U test was used. Statistical significance was considered at $P \leq 0.05$.

Results

The mean values and standard deviation of salivary TNF-α levels were calculated at T0, T1, T2, and T3 in the FA and CA groups.

The results showed that following the placement of the orthodontic appliance, the mean value of the salivary TNF-α level increased and peaked at T1, with values of 51.5 pg/mL and 43.29 pg/mL in the FA and CA groups, respectively, followed by a decline at T2 and T3. Mann–Whitney U test indicated that there were significant differences in salivary TNF-α levels between the two groups at T1, T2, and T3, but the difference was insignificant at T0 [Table 1].

The difference in FA and CA groups between T0 and T1, T0 and T2, T0 and T3, T1 and T2, T1 and T3, and T2 and T3 were statistically significant [Table 2].

Comparing the salivary TNF-α levels at different time points in the FA group using the Wilcoxon matched pairs test showed that differences in the levels between T0 and T1, T0 and T2, T0 and T3, T1 and T2, and T2 and T3 were statistically significant, except between T1 and T3 [Table 3].

Comparing the salivary TNF-α levels at different time points in the CA group using the Wilcoxon matched pairs

Table 1: Mean levels (pg/ml), standard deviation, and coefficient of variation of salivary TNF-α biomarker at T0, T1, T2, and T3 time intervals in FA and CA groups

Time interval	Groups	*n*	Mean TNF-α level (pg/ml)	Standard deviation (SD)	Coefficient of variation	Sum of ranks	Significance (*P*)
T0	FA	20	43.32	7.35	16.96	111.00	0.6502
	CA	20	41.93	6.51	15.52	99.00	
T1	FA	20	51.51	7.38	14.33	139.00	0.0102*
	CA	20	43.29	6.07	14.02	71.00	
T2	FA	20	49.79	7.64	15.34	130.50	0.0500*
	CA	20	42.97	5.96	13.87	79.50	
T3	FA	20	48.68	7.66	15.73	129.00	0.0490*
	CA	20	41.97	6.03	14.37	81.00	

P≤0.05 is considered significant

Table 2: Comparison of FA and CA groups with levels of Salivary TNF alpha biomarker at T0, T1, T2, and T3 intervals by Mann–Whitney *U* test

Time interval	Groups	Mean	Standard deviation (SD)	Sum of ranks	*U*	*Z*	Significance (*P*)
T0-T1	FA	-8.19	2.56	55.00	0.00	-3.7796	0.0002*
	CA	-1.36	0.87	155.00			
T0-T2	FA	-6.47	2.68	56.00	1.00	-3.7041	0.0002*
	CA	-1.04	0.74	154.00			
T0-T3	FA	-5.36	2.57	56.00	1.00	-3.7041	0.0002*
	CA	-0.04	0.72	154.00			
T1-T2	FA	1.72	0.96	149.00	6.00	-3.3261	0.0009*
	CA	0.32	0.52	61.00			
T1-T3	FA	2.83	1.51	140.00	15.00	-2.6458	0.0082*
	CA	1.31	0.65	70.00			
T2-T3	FA	1.11	0.79	109.00	46.00	-0.3024	0.7624
	CA	0.99	0.52	101.00			

P≤0.05 is considered significant

Table 3: Comparison of T0, T1, T2, and T3 time intervals with levels of TNF alpha salivary biomarker in the FA group by Wilcoxon matched pairs test

Time intervals	Mean	Standard deviation	Mean difference	Standard deviation difference	*Z*	Significance (*P*)
T0	43.32	7.35	-8.19	2.56	2.8030	0.0050*
T1	51.51	7.38				
T0	43.32	7.35	-6.47	2.68	2.8031	0.0051*
T2	49.79	7.64				
T0	43.32	7.35	-5.36	2.57	2.8031	0.0051*
T3	48.68	7.66				
T1	51.51	7.38	1.72	0.96	2.8003	0.0058*
T2	49.79	7.64				
T1	51.51	7.38	2.83	1.51	2.8005	0.0059
T3	48.68	7.66				
T2	49.79	7.64	1.11	0.79	2.5992	0.0093*
T3	48.68	7.66				

P≤0.05 is considered significant

test showed that differences in the levels between T0 and T1, T0 and T2, T1 and T2, T1 and T3, and T2 and T3 were statistically significant, except between T0 and T3 [Table 4].

Discussion

Orthodontic tooth movement occurs via the remodeling of the alveolar bone as a result of the force that is exerted on the periodontium. A cell-free hyalinized zone occurs on the pressure side of the periodontal ligament. Necrosis in this zone is mediated by osteoclasts that originate from neighboring tissues.[15] On the tension side, osteoblasts participate in the bone apposition process. The demand for CAs has increased significantly in recent years as they are more comfortable and provide better aesthetics.[19] However, the literature examining bone metabolism with the use of CAs is scarce.

Proinflammatory cytokines such as TNF-α play an important role in bone resorption and root resorption. It

Table 4: Comparison of T0, T1, T2, and T3 time intervals with levels of TNF alpha salivary biomarker in CA group by Wilcoxon matched pairs test

Time intervals	Mean	Standard deviation	Mean difference	Standard deviation difference	*Z*	Significance (*P*)
T0	41.93	6.51	-1.36	0.87	2.8031	0.0051*
T1	43.29	6.07				
T0	41.93	6.51	-1.04	0.74	2.8031	0.0051*
T2	42.97	5.96				
T0	41.93	6.51	-0.04	0.72	0.0510	0.9594
T3	41.97	6.03				
T1	43.29	6.07	0.32	0.52	1.9257	0.0500*
T2	42.97	5.96				
T1	43.29	6.07	1.31	0.65	2.8031	0.0051*
T3	41.97	6.03				
T2	42.97	5.96	0.99	0.52	2.7011	0.0069*
T3	41.97	6.03				

$P \leq 0.05$ is considered significant

involves the recruitment of inflammatory cells and bone resorption through its ability to stimulate Interleukin-1 and granulocyte-macrophage colony-stimulating factor (GM-CSF), inhibit bone collagen synthesis, induce collagenases and stimulate osteoclast differentiation in the presence of GM-CSF.[18]

Lowney *et al*.[11] reported elevation in TNF-α attributed to the orthodontic force. Saadiet *et al*.[19] studied the changes in TNF-α levels in the saliva during orthodontic tooth movement. Their results indicated an increase at 24 h, followed by a decline in the 1st and 2nd weeks, with the highest statistically significant difference. In the present study, too, an increase in salivary TNF-α levels was observed at T1 (24 h). TNF-α is produced primarily by activated monocytes and macrophages but also by osteoblasts and has been proven to be an activator of osteoclastic bone resorption.

Basaran *et al*.[20] explored the changes in TNF-α and interleukin (IL)-1β levels during leveling and distalization in patients undergoing orthodontic treatment. Their findings revealed that baseline levels for the concentrations of TNF-α and IL-1β increased on days 7 and 21 of leveling and distalization. The results from the present study showed that the mean value of salivary TNF-α increased and peaked at T1 after the placement of the orthodontic appliance in both FA and CA groups, followed by a decline at T2 and T3 [Table 1]. This elevation at 24 h might be caused by the early upregulation of chemotactic activities directly after continuous mechanical force application, which agrees with previous studies.[21-23]

Karacay *et al*.[24] examined the changes in TNF-α levels during two different canine distalization techniques (hybrid retractor and rapid canine distalization). Their results showed that heavy force caused a rapid release of TNF-α and that the tissue response continued for a prolonged period. Similarly, in our study, rapid and increased production of TNF-α occurred because FAs cause more pain, pressure, and tension than CAs. The latter exerts intermittent force, allowing tissue reorganization before compressive forces are reapplied.[25]

Conclusion

This study showed that tooth movement in humans is accompanied by an increase in TNF-α levels. In the saliva, TNF-α levels increased significantly during the initial stages of FA and CA treatment at different time points. The mean salivary TNF-α levels in both FAs and CAs increased significantly at 24 h, followed by a decline on the 7th day and the 21st day. Moreover, there were significant differences between FA and CA treatment, with FAs resulting in a significantly higher level of TNF-α in the saliva at different time points compared with CAs.

Authorship declaration

All the authors have contributed significantly to this study and are in agreement with the manuscript.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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