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Evaluation of Salivary Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) in Orthodontic Patients Treated With Clear Aligners vs Conventional Brackets

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Statistical Analysis C
Data Interpretation D
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Background: Total oxidant status (TOS) and total antioxidant status (TAS) can provide information about oxidative damage in tissues. This study aimed to evaluate changes in TOS and TAS in saliva among patients with gingivitis undergoing orthodontic treatment with either conventional brackets (CBs) or clear aligners (CAs) over a 30-day period.

Material/Methods: Thirty patients undergoing non-extraction orthodontic treatment were enrolled and divided into 2 groups (CB group, 15 patients; CA group, 15 patients). Saliva samples were collected at baseline (T0), day 7 (T1), and day 30 (T2) of treatment. Clinical periodontal parameters were recorded, and TAS and TOS levels were assessed using ELISA.

Results: In results, when clinical indices were examined, GI and PI increased, BOP decreased, and PD and CAL showed minimal change between T0 and T2. In saliva samples, TAS and TOS values increase at T1 and then decline toward T2 but remained close to baseline values. Similarly, in the CB group, TAS and TOS values increased at T1 and then decreased toward T2 but remain closed to baseline values. The changes of TAS and TOS at the time intervals within the CA group and CB group were significantly different. Although there was more change in the group CB, no significant difference was found between the groups.

Conclusions: Orthodontic treatment with CAs can cause oxidative tissue stress similar to that with CBs. Given similar oxidative stress data, both treatment methods could be considered for use in orthodontic treatment.

Keywords: Comparative Study • Dental Brackets • Orthodontics • Oxidative Stress • Saliva

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Introduction

Under normal physiological conditions, there is a dynamic balance between reactive oxygen species production and endogenous antioxidant concentration. When this balance is disrupted, either due to excessive reactive oxygen species production or a reduction in endogenous antioxidant defenses, oxidative stress occurs [1-6].

Oxidative stress plays a significant role in tissue damage and is associated with various systemic diseases, such as rheumatoid arthritis, diabetes, AIDS, and cancer [7]. There is also growing evidence that oxidative stress is closely linked to periodontal health [8]. Alterations in antioxidant capacity in saliva and plasma can influence the progression of periodontal conditions such as gingivitis and periodontitis [9].

Dental plaque is the primary etiological factor in the development of gingival inflammation and periodontitis [10]. Orthodontic treatment has been shown to contribute to increased supragingival plaque accumulation and subsequent gingival inflammation [11]. This is largely due to the increased number of plaque-retentive sites and difficulties in mechanical plaque removal during orthodontic therapy [12]. Variability in the design and material properties of orthodontic brackets further influences plaque retention and, consequently, the risk of gingivitis [13]. Certain orthodontic appliances may hinder effective toothbrushing and influence tissue response to tooth movement [14]. In a study by Van Gastel et al [11], bracket design was shown to significantly affect plaque accumulation and periodontal parameters within the first week of appliance placement.

In recent decades, clear aligners (CAs) have gained popularity as a more aesthetic and user-friendly alternative to fixed orthodontic appliances. The Invisalign system, a pioneer in CA technology, uses CAD/CAM systems to produce 3-dimensional treatment models, eliminating the need for repeated physical impressions at each visit [15]. While many brands share fundamental similarities, they can differ in manufacturing techniques and clinical implementation [16-18].

CA therapy offers several advantages, including improved aesthetics, ease of use, shorter appointment durations, reduced discomfort, minimal interference with eating, lower plaque accumulation, and better oral hygiene maintenance [19,20]. Compared with fixed appliances, CA has been associated with a lower risk of dental caries. Therefore, appropriate oral hygiene practices and appliance selection are essential for minimizing treatment-related complications [21].

Given the influence of dental plaque on the balance between reactive oxygen species and endogenous antioxidants, and the potential of CA to promote superior oral hygiene, we aimed to investigate changes in total oxidant status (TOS) and total antioxidant status (TAS) in saliva samples of patients with gingivitis undergoing orthodontic treatment with either conventional brackets (CB) or CA over a 30-day period.

Material and Methods

Study Design and Participants

A total of 30 individuals were included in this study. The CB group consisted of 15 patients (8 women and 7 men) aged between 20 and 24 years (mean age: 21.5±3.4). The CA group (Align Technology, Santa Clara, CA, USA) included 15 patients (7 women and 8 men) aged between 19 and 27 years (mean age: 21.7±3.1).

Ethics approval was obtained from the Ethics Committee of Izmir Katip Çelebi University, and written informed consent was collected from all participants in accordance with the Declaration of Helsinki.

Inclusion Criteria

Eligible participants met the following criteria: no systemic diseases; no medication use within the past 6 months; no smoking or no substance use; good oral hygiene; cooperative and compliant behavior; completion or near-completion of pubertal growth; requirement for orthodontic treatment; and at least 14 permanent teeth present in both the maxilla and mandible.

All participants were diagnosed with gingivitis. Before and during treatment, all patients received standardized oral hygiene instruction at regular intervals.

Study Design and Clinical Procedure

This assessor- and laboratory-blinded controlled clinical study included a total of 30 patients, who were randomized into 2 groups by a coin toss. In the randomization process, the coin toss procedure was performed on each patient by an uninvolved individual who did not know the patients or the procedure to be performed. This individual kept the results confidential after the patient was included in the randomization. The groups were pre-determined based on which side of the coin each participant had been assigned using a sealed-envelope method. After it was determined that the patient met the eligibility criteria, a different individual identified the patient's group using the coin result, and the physician was informed of the allocation.

The 3 researchers in the study knew which group the patients belonged to because they were treating them. Similarly, the patients knew which treatment method was applied to them,

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so neither the patients nor the researchers were blinded in the study. Only the individual performing the laboratory analysis of the data did not know which group the data belonged to.

Saliva samples were collected at 3 time points: before treatment (T0); at 1 week after treatment initiation; and at 1 month after treatment initiation (T2). Clinical periodontal parameters were measured at T0 and T2, and included the plaque index (PI) [22], gingival index (GI) [23], probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP).

All clinical evaluations were performed by a periodontologist (ASE), following standardized protocols. Each patient's oral hygiene routine was assessed, and appropriate techniques were recommended and monitored throughout the study.

Saliva Collection and Volume Measurement

To prevent contamination or fluid loss, all clinical assessments (except PI) were performed after saliva sampling. To obtain an unstimulated saliva sample, the patient was seated upright, the head was tilted forward, and saliva was collected at the floor of the mouth. The saliva thus formed was collected in a plastic container for 15 minutes. Then, it was transferred to a propylene tube with a sterile syringe. The tubes were centrifuged in a centrifuge device for 10 minutes (600 rpm, 500 *g*). Then, the clear part remaining at the top of the propylene tube was taken up a sterile syringe and transferred to a different propylene tube, 0.5 mL in each tube. The tubes were isolated with paraffin tape and stored at -80°C until the day of analysis.

In this study, saliva samples were collected from patients. Saliva samples can vary depending on the time and method of collection, as well as individual differences. To prevent these variations, repeated samples were taken, and the results were divided according to the number of repetitions. When the results were examined, erroneous, excessively high, or excessively low data were excluded from the study.

TAS and TOS Measurements in Saliva

Total antioxidant status (TAS) and total oxidant status (TOS) levels in saliva samples were quantified using commercial ELISA kits (DIAsource ImmunoAssays S.A., Belgium) according to the manufacturer's instructions. Briefly, 100 µL of standard or sample was added to each well and incubated for 1 hour at 37°C. This was followed by the addition of 100 µL of detection antibody, with incubation for 1 hour and washing 3 times. Next, 100 µL of detector solution was added, followed by a 1-hour incubation and 5 wash cycles. Subsequently, 100 µL of substrate solution was added and incubated for 15 to 25 minutes at 37 °C. Finally, absorbance was measured at 450 nm using a microplate reader (BioTek, VT, USA).

Statistical Analysis

Sample size determination was achieved through GPower analysis. (Three studies with compatible data pairs were selected, and their mean and standard deviations were entered to determine the number of patients in each group. The expected effect size was 15, primary outcome was TAS, and according to the studies used, it was predicted that there should be 15 individuals in each group.) With a significance level of 0.05 and a power of 90%, a minimum requirement of 15 patients per group was indicated. Data were analyzed using SPSS version 20.0 (IBM Corp, Armonk, NY, USA). Outliers were defined using the interquartile range (IQR) method (eg, ±3 standard deviations [SD] limits), and only data outside these predefined thresholds were excluded from the analysis. No subjective or post hoc data exclusion was performed. The normality of the clinical and biochemical variables was tested using the Kolmogorov-Smirnov test. When the results of the normality test were considered, it was determined that the PI and GI data were not normally distributed, while the CAL, BOP, and PD data were normally distributed. Parametric data in the study were analyzed using repeated-measures ANOVA, and non-parametric data were analyzed using the Friedman test. Within-group comparisons were analyzed using the Wilcoxon test. Spearman rank correlation was used to evaluate associations between clinical and biochemical variables. In the statistical analysis, baseline data were compared between the 2 groups. Changes after 2 sessions were then compared between groups and against changes after 1 session.

Results

The mean age of participants was 23.44±2.07 years. There were no statistically significant differences between the CA and CB groups in terms of age or sex.

By the end of the first month (T2), GI and PI increased ($P<0.05$), BOP decreased ($P<0.05$), and PD and CAL showed minimal change between T0 and T2. When changes in clinical periodontal indices were examined between the CA and CB groups, no statistically significant differences were found (**Table 1**).

In the CA group, salivary TOS values increased at T1 and then decreased toward T2 but remained close to baseline values. Similarly, in the CB group, salivary TOS values increased at T1 and then decreased toward T2 but remained close to baseline values. These changes in TOS at different time intervals were significant in both groups ($P<0.05$). However, the change in TOS between the CA and CB groups did not differ significantly ($P>0.05$) (**Figure 1, Table 2**).

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Table 1. Clinical periodontal indices (GI, PI, PD, CAL, and BOP) in study groups (clear aligners and conventional brackets) at baseline (T0) and 1 month of orthodontic treatment (T2).

	Clear aligner group		Conventional bracket group		P
	T0	T2	T0	T2	
GI (0-3)	0.69±0.32	1.17±0.07	0.61±0.45	1.19±0.11	(P<0.05)
PI (0-3)	0.82±0.42	1.27±0.21	0.41±0.38	1.29±0.18	(P<0.05)
PD (mm)	2.01±0.34	2.00±0.8	1.89±0.27	2.09±0.26	(P>0.05)
CAL (mm)	2.21±0.7	2.13±0.6	2.10±0.37	2.09±0.17	(P>0.05)
BOP (%)	19±19.5	12.6±15.2	19.5±9.3	12.3±16.0	(P<0.05)

Abbreviations: GI, gingival index; PI, plaque index; PD, probing depth; CAL, clinical attachment level; BOP, bleeding on probing. A linear mixed-effects model was used for within-group comparisons. Between-group comparisons were performed using the Mann-Whitney U test with Bonferroni correction. *P*<0.05 was considered statistically significant.

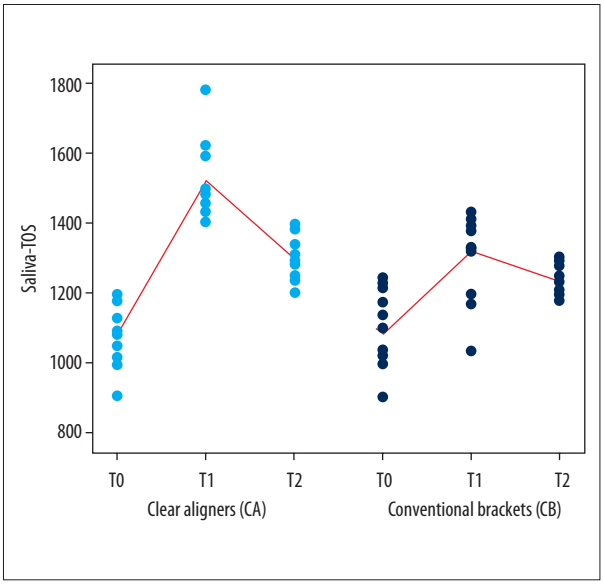

Figure 1. Distribution of saliva total oxidant status (TOS) data (ng/mL) clear aligner (CA) and conventional bracket (CB) groups at before-study baseline (T0), 1 week of orthodontic treatment (T1) and 1 month of orthodontic treatment (T2).

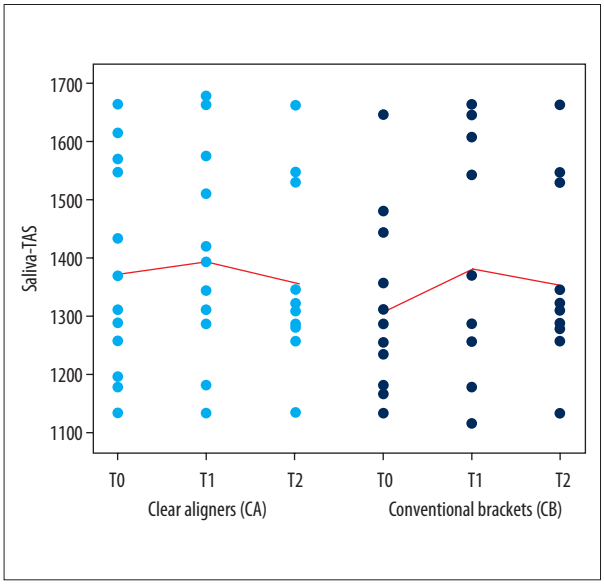

Figure 2. Distribution of saliva total antioxidant status (TAS) data (ng/mL) in clear aligner (CA) and conventional bracket (CB) groups at before-study baseline (T0), 1 week of orthodontic treatment (T1), and 1 month of orthodontic treatment (T2).

In the CA group, salivary TAS values increased at T1 and then decreased toward T2 but remained close to baseline values. Similarly in the CB group, salivary TAS values increased at T1 and then decreased toward T2 but remained close to baseline values. These changes in TAS at different time intervals were significantly different in *(P*<0.05). When changes in TAS were compared between the CA and CB groups, no significant difference was observed *(P*>0.05) (**Figure 2, Table 2**).

When correlations between TAS and TOS (T2-T0) and BOP (T2-T0) were analyzed, TAS and TOS values increased as BOP increased and decreased as BOP decreased. A significant positive correlation was observed between TOS and BOP (*P*<0.05). When changes between time points (T2-T0) were examined, increases in salivary TOS were associated with increases in BOP (*P*<0.05). Although a positive correlation was observed between TAS and BOP, this association was not statistically significant (*P*>0.05) (**Table 3**).

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Table 2. Distribution of saliva total oxidant status (TOS) and total antioxidant status (TAS) data in clear aligner and conventional bracket groups at baseline (T0), 1 week of orthodontic treatment (T1), and 1 month of orthodontic treatment (T2).

	Clear aligner group			Conventional bracket group			P
	T0	T1	T2	T0	T1	T2	
TAS ng/mL	1368±179[a]	1402±394[b]	1351±253[a]	1318±185[c]	1380±158[d]	1355±236[c]	(P_1<0.05) (P_2>0.05)
TOS ng/mL	1166±141[a]	1555±142[b]	1390±622[a]	1175±172[c]	1401±166[d]	1362±926[c]	(P_1<0.05) (P_2>0.05)

When different superscript letters are present, values differ significantly between groups. P_1: comparison of within-group changes over time. P_2: comparison between groups. P<0.05 indicates a statistically significant difference between groups. Statistical analysis: Linear mixed-effects model was used for within-group comparisons. Between-group comparisons were performed using the Mann-Whitney U test with Bonferroni correction.

Table 3. Correlation between bleeding on probing and saliva total oxidant status and total antioxidant status data.

			Bleeding on probing (T2-T0)	Total antioxidant status (T2-T0)	Total oxidant status (T2-T0)
Spearman rho	Bleeding on probing (T2-T0)	Correlation coefficient P (2-tailed) n		0.068 0.583 30	0.283 0.042 30
	Total antioxidant status (T2-T0)	Correlation coefficient P (2-tailed) n	0.068 0.583 30		0.029 0.755 30
	Total oxidant status (T2-T0)	Correlation coefficient P (2-tailed) n	0.283 0.042 30	0.029 0.755 30	

P<0.05: The groups are statistically different from each other. Baseline (T0), 1 month of orthodontic treatment (T2).

Discussion

Recent advancements in orthodontics have increasingly focused on aesthetics, reflecting a growing demand across all age groups for treatment options that not only correct dental malocclusions but also enhance appearance. As a result, many patients now seek orthodontic interventions that are visually discreet and comfortable. This demand has led to the development of more aesthetically pleasing appliances, such as CAs, which are preferred by individuals who are concerned about the visibility of traditional brackets during treatment [24-26]. Patients with aesthetic concerns often reject conventional fixed appliances due to the visibility of metal components [27].

The pursuit of aesthetic and comfortable orthodontic solutions has led to the introduction of various alternatives to metal brackets, including ceramic, plastic, polycarbonate, vinyl, zirconia, and Teflon-coated brackets [28]. However, despite their tooth-colored appearance, many patients still find these alternatives unsatisfactory. Consequently, attention has shifted to more discreet treatment modalities, such as lingual brackets and CAs, which are less visible and can be easily inserted and removed by patients. These systems have been well-received by patients concerned with dental aesthetics, contributing to increased self-confidence and treatment compliance [29].

CA treatment offers several advantages, including enhanced aesthetics, ease of use, shorter appointment durations, reduced pain and discomfort, improved eating convenience, and better facilitation of oral hygiene [30,31]. Given these benefits, our study compared CA and CB treatment modalities, not only in terms of appearance but also in their effects on periodontal health and oxidative stress. The results support the hypothesis that CA treatment leads to less oxidative damage while maintaining improved aesthetic outcomes.

Orthodontic forces applied during tooth movement lead to compression and tension within the periodontal ligament, alterations in the extracellular matrix, and localized tissue injury. These events trigger vascular changes, such as vasodilation and increased capillary permeability, culminating in aseptic inflammation characterized by the release of inflammatory

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mediators, cytokines [29,30]. Cytokines act as signaling proteins between immune cells and play a critical role in periodontal remodeling through cell activation, proliferation, and apoptosis during tooth movement [31].

Studies conducted in vivo and in vitro have demonstrated that cytokines are involved in bone resorption and deposition, highlighting their importance in orthodontic bone remodeling. These cytokines also appear to increase in the early stages of orthodontic treatment as part of the inflammatory response [31]. Periodontal inflammation, often exacerbated by increased plaque accumulation associated with orthodontic appliances, also contributes to elevated TAS and TOS levels in the saliva [32].

Oxidative stress has been implicated in the pathology of numerous systemic diseases and is commonly measured in biological fluids, such as urine, blood, and saliva [30]. In dentistry, particularly periodontology, the relationship between oxidative stress and periodontal inflammation has been widely studied. However, in orthodontics, existing studies have largely focused on in vitro assessments of oxidative stress induced by appliance materials. To date, no in vivo human studies have specifically evaluated oxidative damage or inflammation associated with CA use, making our study novel in this regard.

Certain conventional orthodontic techniques may apply greater force or lead to higher plaque accumulation, both of which can intensify inflammation and cytokine secretion. These conditions are particularly associated with the use of fixed brackets. In contrast, CAs tend to provoke a milder biological response. TAS and TOS salivary data have also yielded similar results in comparable patient groups in studies using gingival crevicular fluid [33]. Different types of materials are used in orthodontic treatments. Especially in the use of CBs, different orthodontic and periodontal responses can be obtained depending on the type of bracket. Different brackets affect patients' quality of life in different ways [34]. In our study, CBs were compared with CAs, and CAs were found to be more comfortable. In some orthodontic treatments, complications can also be observed, and the rate of complications can increase depending on the materials used. The type of bracket and the type of orthodontic treatment may be determining factors in the development of complications [35]. In our study, complications were not evaluated; however, these complications may be investigated in future studies.

To our knowledge, sufficient studies in the literature have evaluated TAS and TOS levels in saliva during CA treatment, marking our work as the first to explore this relationship. Nonetheless, the study has limitations, including a relatively small sample size, the absence of systemic (eg, blood) TAS and TOS measurements, and the lack of additional biomarkers to assess inflammation and oxidative damage. Also, baseline PI values differed between groups (CA, PI=0.82; CB, PI=0.41), which may influence longitudinal comparisons.

Conclusions

The marked increase in TOS and TAS levels observed during the first week of orthodontic treatment, followed by a return to nearly baseline levels after 1 month, supports our hypothesis that free radical production in the periodontium remains within physiological limits when CA and CB are used. An initial spike in oxidative stress at T1 was observed, consistent with the initiation of tooth movement, followed by a recovery at T2. This finding suggests that tooth movement achieved with CA and CB treatments, along with the associated aseptic inflammatory short-term biological response, did not result in oxidative damage. Therefore, for patient populations at higher risk of oxidative damage, CA may be a preferable alternative to conventional fixed orthodontic appliances. However, further research is needed to fully elucidate the long-term biological effect of CA-based orthodontic treatment on periodontal and systemic tissues.

Ethics Approval and Consent to Participate

The present study was analyzed and approved by the Izmir Katip Celebi University Human Ethics Research Committee.

Availability of Supporting Data

The datasets used and/or analyzed during the present study are availablefrom the corresponding author on reasonable request.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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