

**CHANGES IN SALIVARY 8-ISO-PROSTAGLANDIN F2 α
AFTER SIX MONTHS OF ORTHODONTIC APPLIANCE
WEAR: A PROSPECTIVE PAIRED STUDY**

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Abstract

Orthodontic appliances may influence oral redox homeostasis through a combination of mechanical forces, biofilm alterations, inflammatory responses, and long-term exposure to dental materials and corrosion products. These factors may contribute to oxidative stress within the oral cavity, particularly during the initial phases of orthodontic treatment. F2-isoprostanes, and especially 8-iso-prostaglandin F2 α (8-iso-PGF2 α), are considered reliable biomarkers of lipid peroxidation and oxidative stress in vivo. Saliva represents a practical and non-invasive biological matrix for monitoring local biochemical changes associated with orthodontic therapy [1–5].

The aim of the present study was to evaluate changes in salivary 8-iso-PGF2 α levels before orthodontic appliance placement (T0) and six months after placement (T1) in patients undergoing orthodontic treatment.

A prospective paired longitudinal study was conducted in patients receiving orthodontic appliances. A total of 137 participants provided baseline (T0) saliva samples, and 122 completed the 6-month follow-up with paired samples at T0 and T1. The planned one-month follow-up could not be included due to participant attrition associated with repeated saliva sampling and technical failure of the analytical equipment. Consequently, the present analysis focuses on paired baseline and six-month measurements. Unstimulated whole saliva was collected under standardized conditions. Salivary 8-iso-PGF2 α concentrations

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were quantified using liquid chromatography–tandem mass spectrometry (LC–MS/MS) with selective reaction monitoring and internal standardization, following salt-assisted protein precipitation and liquid–liquid extraction [6]. Values below the analytical limit of detection were imputed as half the detection limit (LOD/2, 0.5 pg/mL). Data are presented as median and interquartile range (IQR). Paired comparisons were performed using the Wilcoxon signed-rank test, and effect size was calculated as $r = |z|/\sqrt{n}$. Median salivary 8-iso-PGF2 α concentration decreased from 6.42 pg/mL (IQR 4.23–10.14) at baseline to 4.60 pg/mL (IQR 2.94–7.05) six months after appliance placement. The reduction was statistically significant (Wilcoxon $W = 1891$, $p < 0.001$) and was associated with a moderate effect size ($r = 0.43$). In the paired cohort ($n = 122$), 35% were males and 65% females, with ages ranging from 8 to 63 years. Most participants (85/122; 69.7%) were treated with appliances containing metal elements. The decrease in 8-iso-PGF2 α remained statistically significant within age- and sex-based subgroups.

Salivary 8-iso-prostaglandin F2 α concentrations were significantly reduced six months after orthodontic appliance placement compared with baseline. These findings indicate a decrease in oral lipid peroxidation over time. Measurement of salivary 8-iso-PGF2 α by LC–MS/MS represents a reliable and non-invasive approach for monitoring oxidative stress in orthodontic patients.

Key words: saliva, oxidative stress, 8-iso-prostaglandin F2 α , isoprostanes, orthodontics, LC–MS/MS

Introduction. Oxidative stress reflects an imbalance between reactive oxygen species (ROS) and antioxidant defenses, leading to oxidative modification of biomolecules in vivo [1]. Lipid peroxidation is a key downstream consequence, and F2-isoprostanes – particularly 8-iso-prostaglandin F2 α (8-iso-PGF2 α) – are considered reliable markers due to their chemical stability and suitability for mass-spectrometric quantification [2,3]. Although enzymatic pathways may contribute under inflammatory conditions, 8-iso-PGF2 α remains robust when interpreted in relation to oral inflammatory status and sampling time points [4]. Saliva is a practical, non-invasive matrix for monitoring oral biochemical changes when collection and pre-analytical handling are standardized [5]. Orthodontic treatment may influence oral redox balance via tooth-movement-related inflammatory signalling, appliance-related plaque retention and gingival changes, and documented ion release from components (e.g., nickel, chromium), while published findings remain heterogeneous due to differences in biomarkers and follow-up schedules [7–12]. Given matrix effects in saliva, LC–MS/MS provides high specificity and sensitivity for isoprostanes [6,13,14]; therefore, this prospective paired study examined changes in salivary 8-iso-PGF2 α before and six months after orthodontic appliance placement using LC–MS/MS. The aim of the present study was to evaluate changes in salivary 8-iso-PGF2 α levels before orthodontic appliance placement (T0) and six months after placement (T1) in patients undergoing orthodontic treatment.

Materials and methods. Study design and participants. This prospective longitudinal study included saliva sampling before orthodontic appliance placement (T0) and at six months (T1). Eligibility criteria were defined a priori. Participants were clinically healthy at inclusion, without acute systemic illness or active oral inflammatory conditions at sampling. Exclusion criteria included antibiotic therapy within one week prior to saliva collection and the use of antioxidant-containing supplements. Participants were instructed to maintain their usual diet and oral hygiene habits during follow-up, acknowledging that fixed appliances may affect oral hygiene efficacy. All samples were processed in the university laboratory under standardized conditions, using the same LC–MS/MS instrumentation, internal standardization, and calibration approach across the dataset to minimize analytical variability.

Saliva collection and pre-analytical standardization. Sampling was standardized to reduce pre-analytical variability. All specimens were collected between 09:00 and 12:00 to limit circadian effects. Participants refrained from eating and drinking (except water) and avoided tooth brushing for 2–3 h prior to collection, then rinsed thoroughly with water immediately before sampling. Unstimulated whole saliva was collected by passive drooling/expectorations into sterile polypropylene containers, labelled with patient identifiers and time point. Samples were promptly frozen at -20°C and stored until analysis. Prior to LC–MS/MS processing, samples were thawed under controlled conditions and gently mixed for homogeneity; the same workflow was applied to T0 and T1 samples.

LC–MS/MS quantification of 8-iso-PGF 2α . Quantification of 8-iso-PGF 2α in saliva was performed using a validated LC–MS/MS method [6]. Proteins were removed by salt-assisted precipitation, followed by liquid–liquid extraction with ethyl acetate to reduce matrix interferences. Chromatographic separation was achieved on a solid-core analytical column, and detection was performed on a triple quadrupole mass spectrometer in selective reaction monitoring mode. Quantification used an external calibration curve with internal standard correction to account for extraction variability and matrix effects; analytical linearity followed the validated approach [6]. LC–MS/MS offers high selectivity for 8-iso-PGF 2α relative to immunoassay-based methods, which may be susceptible to cross-reactivity [2, 3, 6, 13, 14].

Handling of values below the detection limit. One follow-up sample was reported as below the analytical limit of detection (< 1.0 pg/mL) and was imputed as 0.5 pg/mL (LOD/2) to preserve the paired dataset and avoid exclusion of low but plausible concentrations.

Statistical analysis. Continuous variables were assessed for distribution using graphical inspection and the Shapiro–Wilk test. As 8-iso-prostaglandin F 2α values were right-skewed, results are presented as median and interquartile range (IQR). Paired comparisons between T0 and T1 were performed using the Wilcoxon signed-rank test, with each participant serving as their own control. Effect size

was calculated as $r = |z|/\sqrt{n}$, where n is the number of paired observations. Analyses were conducted in IBM SPSS Statistics v22 (IBM Corp., Armonk, NY, USA); $p < 0.05$ (two-tailed) was considered statistically significant.

Results. Descriptive characteristics of the study sample. A total of 137 patients were initially enrolled and provided baseline (T0) saliva samples. Of these, 122 participants completed the 6-month follow-up and provided paired saliva samples at both time points (T0 and T1). Only patients with complete paired observations ($n = 122$) were included in the comparative statistical analysis to preserve the validity of the paired design. Detailed demographic characteristics, including sex distribution, are presented in Table 1. Age characteristics are summarized in Table 2. Regarding appliance composition, 85 of 122 participants (69.7%) had orthodontic appliances with metal elements, while 37 (30.3%) were treated with appliances without metal elements (Table 3).

T a b l e 1

Sex distribution of the study population

Sex	Total enrolled patients ($n = 137$)	Patients with paired samples ($n = 122$)
Male	48 (35.0%)	45 (36.9%)
Female	89 (65.0%)	77 (63.1%)
Total	137 (100%)	122 (100%)

T a b l e 2

Age characteristics

Age (years), mean \pm SD	23.56 \pm 14.77
Median (IQR)	17 (13–31)
Range (min–max)	8–63
Age group 8–18 years, n (%)	66 (54.1%), mean \pm SD = 12.79 \pm 2.65
Age group \geq 19 years, n (%)	56 (45.9%), mean \pm SD = 36.25 \pm 12.98

T a b l e 3

Presence of metal elements in orthodontic appliances (paired cohort, $n = 122$)

Metal elements present	85 (69.7%)
No metal elements	37 (30.3%)

Changes in salivary 8-iso-PGF2 α concentrations. At baseline (T0), the median salivary 8-iso-PGF2 α concentration was 6.42 pg/mL (IQR: 4.23–10.14). At follow-up (T1), the median concentration decreased to 4.60 pg/mL (IQR: 2.94–7.05). The median paired difference (T1–T0) was –1.56 pg/mL (IQR: –4.92 to 0.19), indicating an overall reduction over time. The difference between T0 and T1 was statistically significant according to the Wilcoxon signed-rank test ($W = 1891$, $p < 0.001$), with a moderate effect size ($r = 0.43$).

Subgroup analyses by age and sex. To address potential demographic effects, subgroup analyses were performed by age group (8–18 years vs. ≥ 19 years) and by sex. Within each subgroup, paired changes from T0 to T1 were statistically significant (Table 4). These subgroup analyses were considered exploratory.

T a b l e 4

Salivary 8-iso-PGF2 α concentrations by subgroup (paired T0–T1)

Subgroup	n	T0 median (IQR)	T1 median (IQR)	Δ (T1 – T0) median (IQR)	Wilcoxon W	p -value	Effect size r
Age 8–18 years	66	6.48 (4.21–9.59)	4.47 (2.89–6.09)	–2.17 (–4.79 to –0.05)	479	<0.001	0.49
Age ≥ 19 years	56	6.42 (4.47–13.52)	5.08 (3.25–7.77)	–1.34 (–4.98 to 0.23)	472	0.008	0.36
Female	77	6.42 (4.15–10.09)	4.39 (2.90–6.50)	–1.99 (–4.93 to 0.11)	700	<0.001	0.46
Male	45	6.55 (4.53–10.16)	5.02 (3.09–7.09)	–1.45 (–4.42 to 0.55)	308	0.017	0.35

Discussion. Interpretation of the biomarker trajectory. This prospective paired study demonstrated a significant reduction in salivary 8-iso-PGF2 α concentrations six months after orthodontic appliance placement, indicating lower lipid peroxidation at follow-up compared with baseline. As 8-iso-PGF2 α is a robust lipid peroxidation marker and was quantified by LC–MS/MS, the observed decrease is unlikely to reflect analytical cross-reactivity and more plausibly represents a true biological change in the oral redox milieu over time [2, 3, 6, 13, 14]. A plausible interpretation is that early mechanical and inflammatory responses after appliance placement may attenuate with tissue accommodation and improved plaque control, resulting in a lower oxidative burden at later treatment stages. Overall, these findings support a time-dependent oxidative response during orthodontic treatment rather than persistent elevation in clinically healthy individuals.

Comparison with the orthodontic oxidative stress literature. Prior studies on oxidative stress during orthodontic treatment report mixed findings, likely due to differences in biomarkers, sample types, and follow-up timing [7–10]. Early post-placement increases have been linked to transient inflammatory/mechanical responses and plaque-control challenges, whereas later follow-ups more often show stabilization or reductions, consistent with time-dependent redox adaptation in otherwise healthy patients [7–10]. In this context, our LC–MS/MS-based assessment of salivary 8-iso-prostaglandin F2 α (a lipid peroxidation marker) showed a decrease from baseline to six months, which fits a trajectory of

early perturbation followed by stabilization/adaptation; comparisons with studies using other oxidative domains (e.g., antioxidant capacity) remain limited due to non-equivalent endpoints and assay principles [7–10].

Potential role of dental materials and metal ion release. Most participants in the paired cohort had metal-containing orthodontic components (85/122; 69.7%), making material-related effects relevant for interpretation. Nickel and chromium release from orthodontic appliances, including detection in saliva, has been reported [11,12] and may contribute to oxidative or inflammatory signalling, although release kinetics and mucosal tolerance can change over time, potentially attenuating biological effects with prolonged exposure. In parallel, appliances may transiently worsen plaque retention and gingival inflammation early after placement; therefore, the lower 8-iso-PGF2 α at six months may reflect combined tissue accommodation, stabilization of gingival conditions, improved plaque control, and reduced acute material-related responses.

Clinical interpretation and relevance. Clinically, the reduction in salivary 8-iso-PGF2 α at six months suggests that, in clinically healthy patients, orthodontic treatment is not associated with persistently increased lipid peroxidation in saliva. This finding may support patient counselling, particularly for individuals concerned about long-term biological effects of orthodontic materials and chronic inflammation. The study does not exclude short-term oxidative fluctuations early after appliance placement; rather, it indicates that at six months the oxidative status, as reflected by this biomarker, may be lower than baseline.

Limitations. This study assessed salivary 8-iso-PGF2 α at baseline and six months only; thus, early and intermediate biomarker dynamics after appliance placement were not captured. The lack of a non-orthodontic control group and the absence of systematically recorded diet and quantitative oral hygiene/periodontal indices may have introduced unmeasured confounding. Although LC–MS/MS is highly specific, pre-analytical factors (e.g., storage duration, freeze–thaw cycles) could have influenced analyte stability.

Clinical implications and future directions. These findings support salivary 8-iso-PGF2 α as a practical non-invasive biomarker for monitoring oxidative stress dynamics during orthodontic treatment. Future studies should include additional early time points (e.g., one week and one month), incorporate clinical periodontal/plaque indices, and add a control group to strengthen causal interpretation. Integrating oxidative biomarkers with measures of metal ion release and microbial shifts, and examining interindividual variability, may clarify mechanisms and identify subgroups with distinct oxidative trajectories [10–12, 15].

Conclusions. Salivary 8-iso-PGF2 α concentrations were significantly reduced six months after orthodontic appliance placement compared with baseline, indicating decreased oral lipid peroxidation over time. LC–MS/MS quantification of salivary 8-iso-PGF2 α provides a reliable, non-invasive approach for monitoring oxidative stress in orthodontic patients.

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