

Effect of fixed orthodontic appliances on nonmicrobial salivary parameters

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ABSTRACT

Objectives: To examine possible changes in the levels of salivary antioxidants, C-reactive protein (CRP), cortisol, pH, proteins, and blood in patients treated with fixed orthodontic appliances.

Materials and Methods: Salivary samples from 21 orthodontic patients who met specific inclusion criteria were collected before the beginning of orthodontic treatment (T0; baseline), 1 hour after bonding (T1), and 4–6 weeks after bonding (T2). Oxidant-scavenging ability (OSA) was quantified using a luminol-dependent chemiluminescence assay. Cortisol and CRP levels were measured using immunoassay kits. pH levels and presence of proteins and blood in the samples were quantified using strip-based tests.

Results: A significant decrease in salivary pH was observed after bonding ($P = .013$). An increase in oxidant-scavenging abilities during orthodontic treatment was detected, but the change was not statistically significant. Cortisol and CRP levels slightly increased after bonding, but the difference was small without statistical significance. Changes in the presence of proteins and blood were also insignificant.

Conclusions: Exposure to fixed orthodontic appliances did not show a significant effect on salivary parameters related to inflammation or stress, with the exception of a significant but transient pH decrease after bonding. (*Angle Orthod.* 2018;88:806–811.)

KEY WORDS: Antioxidants; Salivary parameters

INTRODUCTION

The oral cavity is a highly complex environment, where multiple synergistic and antagonistic interactions take place at any given time among its constituents.¹ The key players in this environment are saliva secreted by glands, a large variety of microorganisms, plasma agents delivered via the crevicular

fluid, nutrients rich in antioxidant polyphenols, blood cells extravasated during capillary injury, xenobiotics, common drugs, and, in many cases, also proinflammatory agonists generated by leukocytes during infectious and inflammatory episodes.^{2,3}

Fixed orthodontic appliances introduce an additional constituent that may enhance the complexity of the oral environment in a variety of ways. Orthodontic treatment is associated with inflammation: orthodontic tooth movement is performed by a biological process known as sterile inflammation,⁴ and fixed orthodontic appliances may impede the maintenance of excellent oral hygiene, frequently leading to gingivitis.⁵ Therefore, it was of interest to study whether exposure to fixed orthodontic appliances is associated with changes in salivary inflammatory agents, such as C-reactive protein (CRP; an inflammatory marker) and blood. In addition, since salivary protein and albumin levels are known to increase in individuals with gingivitis,⁶ we intended to study whether fixed orthodontic appliances would affect protein levels.

Orthodontic treatment is also accompanied by stress⁷; hence, it was of interest to study whether exposure to fixed orthodontic appliances had an effect on the levels of salivary cortisol.

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Orthodontic appliances are frequently associated with dietary changes because of pain associated with mastication⁸ and instructions given by the orthodontist.⁹ Antioxidant status in the oral cavity is under a constant balance due to synergistic interactions among salivary antioxidants, oral microbial flora, polyphenols from nutrients, and blood elements extravagated from injured capillaries.¹⁰ Therefore, it was of interest to study whether exposure to fixed orthodontic appliances might impact that balance and affect the salivary oxidant-scavenging ability (OSA) and pH.

The aim of the present study was to examine possible changes in the levels of salivary antioxidants, CRP, cortisol, pH, proteins, and blood in subjects exposed to fixed orthodontic appliances. The hypothesis was that orthodontic treatment with fixed appliances would affect these nonmicrobial salivary parameters.

MATERIALS AND METHODS

This was a prospective, self-controlled study. A flowchart of the study design is shown in Figure 1. The study group consisted of patients about to undergo orthodontic treatment with fixed appliances in the Department of Orthodontics, The Hebrew University–Hadassah School of Dental Medicine, Jerusalem, Israel. The inclusion criteria were patients who (1) were free of systemic diseases, (2) had never undergone orthodontic treatment, (3) were free of congenital craniofacial anomalies, (4) were free of allergies, and (5) were not receiving medications. To minimize the number of unknown variables, the same patients, before commencing orthodontic treatment, were used as controls.

Informed consent was obtained from all participants or their parents after a detailed explanation of the study. Ethical approval was obtained from the Hadassah Medical Center ethics committee for clinical trials (0313-11-HMO).

Patients received oral hygiene instructions and were referred to a dental hygienist for cleaning before treatment. All patients were instructed to brush their teeth and tongue 1 hour before saliva collection and to refrain from eating during the following hour. Orthodontic treatment consisted of full bonding of 0.022 × 0.025-inch slot preadjusted edgewise brackets and insertion of 0.014-inch Nitinol archwire. Salivary samples were collected at three time points: before the beginning of orthodontic treatment (T0; baseline), 1 hour after bonding (T1), and 4–6 weeks after bonding (T2). The spitting technique of unstimulated whole saliva, which is considered the simplest method for collection of oral fluids,¹¹ was chosen for this study. Each patient collected 2–4 mL of saliva directly into a test tube. The daily time of collection for the same patient was kept identical to avoid changes in cortisol

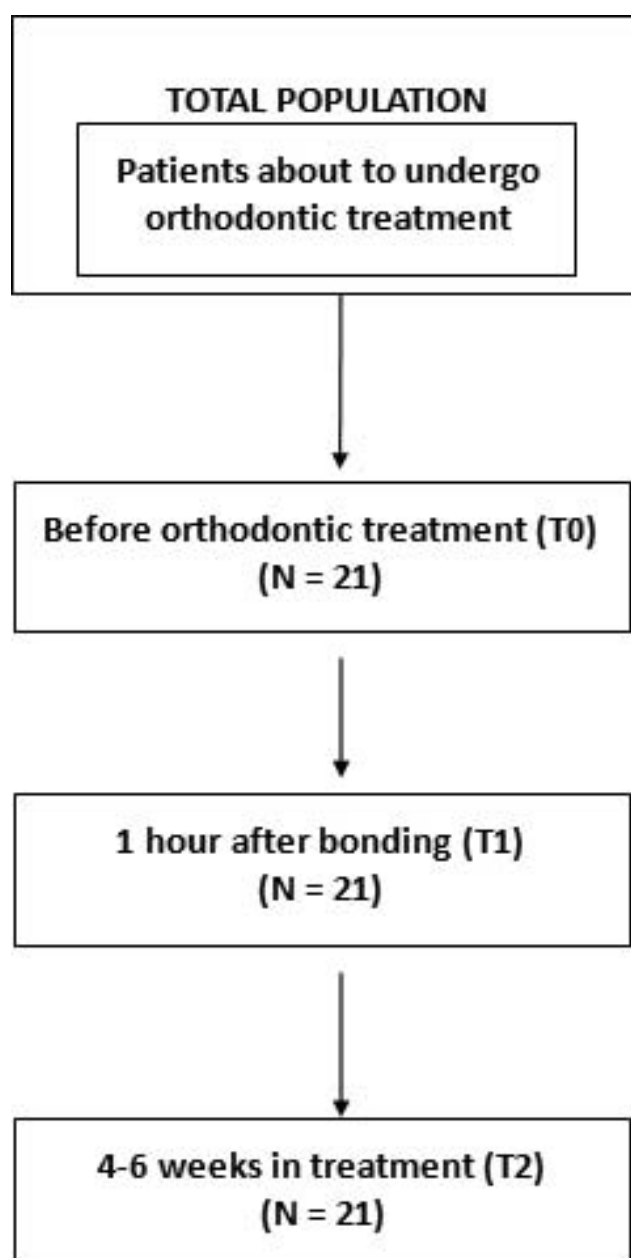


Figure 1. Flowchart of the examined patients.

levels. The time frame of salivary collection was between 10:00 AM and 2 PM, due to the circadian rhythm of cortisol.¹¹ Collected undiluted saliva samples were kept on ice and centrifuged at 10,000 rpm for 5 minutes, and the supernatant fluid was frozen (−20 °C) until analysis. The outcome measures were salivary OSA, cortisol levels, CRP levels, pH levels, and the presence of proteins and blood.

Sample Size Calculation

The required sample size was calculated before the study to minimize the probability of type I and type II

error.¹² The significance level was set at .05 and the power at .8. The minimum sample size required was 17 subjects.

Protocol of Testing and Analysis

A luminol-dependent chemiluminescence (LDCL) assay was used to quantify salivary OSA. Thirty microliters of saliva was used for each experiment. An H₂O₂ cocktail was employed,^{13,14} which was composed of 800 µL of Hanks balanced salt solution at pH 7.4, luminol (10 µM), H₂O₂ (1 mM), sodium selenite (2 mM), and CoCl₂·6H₂O (10 µM). This cocktail generated a constant luminescence light wave due to peroxide and hydroxyl radical. The degree of light quenching in the different samples indicated their OSA.³

Cortisol tests were performed using a salivary cortisol enzyme immunoassay kit based on the Salimetrics enzyme-linked immunosorbent assay (ELISA) test (Carlsbad, CA). Twenty-five microliters of saliva was used for each experiment. A microtiter plate coated with monoclonal antibodies to cortisol was used, in which cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for the antibody binding sites. After incubation, unbound components were washed away. Bound cortisol peroxidase was measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine (TMB), known to produce a blue color. A yellow color was formed after stopping the reaction with sulfuric acid. Optical density was read on a standard plate reader at 450 nm. The amount of cortisol peroxidase thus detected was inversely proportional to the amount of cortisol present.

The CRP tests were performed using a microchip kit based on the Salimetrics enzyme-linked immunoassay. Fifteen microliters of saliva was used for each experiment. A microtiter plate coated with mouse antibodies to human CRP was used. CRP in standards and unknowns and goat anti-human CRP antibodies linked to horseradish peroxidase were added. In this assay, a "sandwich" was formed with the precoated antibody on the bottom, the CRP in the middle, and the antibody linked to horseradish peroxidase on top. After incubation, unbound components were washed away. Bound CRP peroxidase was measured by the reaction of the peroxidase enzyme on TMB. This reaction produced a blue color. A yellow color was formed after stopping the reaction. Optical density was read on a standard microplate reader at 450 nm. The amount of CRP peroxidase detected this way was directly proportional to the amount of CRP present.

Levels of proteins, blood, and pH were measured using Combi-Screen 10SL strips. The protein test is

based on the "protein error" principle of the indicator. A ladder of colors indicated whether the level of protein was absent or below 0.3, 1.0, or 5.0 g/L. The test is especially sensitive in the presence of albumin. Other proteins were indicated with lesser sensitivity. Detection of blood was based on the pseudoperoxidative activity of hemoglobin and myoglobin, which catalyzed the oxidation of an indicator by an organic hydroperoxide and a chromogene producing a green color. While intact erythrocytes were indicated by punctual colorations on the test pad, hemoglobin and myoglobin were indicated by a homogeneous green coloration. The pH test paper contained indicators that clearly changed color between pH 5 and pH 9 (from orange to green to turquoise). The color fields of the present test corresponded to pH values of 5, 6, 6.5, 7, 8, and 9.

Statistical Analysis

Statistical analysis was performed using SPSS software version 22 (IBM SPSS Statistics, Armonk, NY). Means, standard deviations, number of observations, and 95% confidence intervals were calculated for each group, respectively. The distribution of data was tested using Kolmogorov-Smirnov and Shapiro-Wilk tests. Friedman test was used to evaluate overall significance among continuous variables (OSA, pH, cortisol, and CRP). When the Friedman test yielded a significant result, the Wilcoxon signed ranks test was used for comparisons between individual groups. Categorical variables (presence of protein or blood) were analyzed using Cochran's Q test. A two-tailed *P* value <.05 was considered statistically significant.

RESULTS

The study group consisted of 21 orthodontic patients, 14 males and 7 females, with a mean age of 15.8 ± 4.4 years. The control group consisted of the same patients before the beginning of their orthodontic treatment. Salivary samples were collected from all patients at three time points. Table 1 shows the average levels of OSA, pH, cortisol peroxidase, and CRP in the patients' saliva before and during orthodontic treatment. Counts per minute averages of the LDCL assay were used to assess the OSA in the samples. There was a reduction in the OSA during orthodontic treatment, which represents an increase in the oxidant scavenging abilities, but the difference was not statistically significant. There was a significant decrease in the salivary pH after bonding ($P = .013$). Cortisol and CRP levels slightly increased after bonding, but the difference was small without statistical significance.

Table 2 compares the prevalence of blood and proteins in the saliva of the patients. After bonding,

Table 1. Descriptive Statistics: Comparison of Average Levels of OSA, pH, Cortisol Peroxidase, and CRP in Patients' Saliva Before and During Orthodontic Treatment^a

Salivary Parameter	n	Time	Mean	SD	P Value
OSA, cpm	21	T0	179	67	.95
		T1	170	66	
		T2	160	84	
pH	21	T0	6.9	0.2	.013*
		T1	6.69	0.29	
		T2	6.83	0.33	
Cortisol peroxidase, OD	21	T0	1.32	0.25	.74
		T1	1.37	0.25	
		T2	1.33	0.23	
CRP, OD	21	T0	0.15	0.06	.79
		T1	0.16	0.06	
		T2	0.13	0.03	

^a cpm indicates counts per minute; OD, optical density; T0, before treatment (baseline); T1, 1 hour after bonding; T2, 4–6 weeks after bonding.

* $P < .05$.

there was an increase in the prevalence of blood and proteins in the saliva. The increase in blood remained during orthodontic treatment but was not statistically significant. The increase in proteins was transient and returned to its baseline during orthodontic treatment.

DISCUSSION

Fixed orthodontic appliances frequently interfere with good oral hygiene maintenance, leading to plaque accumulation.^{15–17} They also may influence the colonization of periopathogens and other bacteria, resulting in inflammation and bleeding.¹⁸ It therefore seemed likely that these changes would affect nonmicrobial salivary parameters related to stress and inflammation. This study was designed to evaluate this hypothesis.

To minimize the influence of variables such as age and gender, a prospective study was designed with the same subjects before orthodontic treatment as controls. Most previous studies used a similar design,^{19–22} while others used independent control groups.²³

This was the first study to examine the antioxidant capacity in orthodontic patients. Previous studies tested antioxidant levels in the saliva of patients having inflammatory diseases (peri-implantitis or periodontitis) and found a decrease in their oxidative capacity.^{24–26} In contrast, the current patients did not have preexisting inflammatory diseases. Nonetheless, a similar effect due to the known inflammatory effect of orthodontic appliances was expected. The results indeed showed a decrease in the OSA, but it did not reach statistical significance.

For the same reason, an increase in salivary cortisol levels following bonding was also expected, and an increase was observed. However, the difference was small, without statistical significance. Salivary cortisol

Table 2. Comparison of Prevalence of Blood and Proteins in Patients' Saliva Before and During Orthodontic Treatment^a

Salivary Parameter	n	Time	Mean	Cochran's Q Test	P Value
Blood, %	21	T0	81	0.33	.85
		T1	86		
		T2	86		
Proteins, %	21	T0	71	1.6	.45
		T1	81		
		T2	71		

^a T0 indicates before treatment (baseline); T1, 1 hour after bonding; T2, 4–6 weeks after bonding.

levels are known to depend on several other variables such as body weight, protocol design, and technical aspects relating to assay conditions.²⁷ According to a recent study, the method that was used to measure salivary cortisol (enzyme immunoassay kit) was found to be reliable.²⁸

The inflammation anticipated during orthodontic treatment should also be observed in inflammatory markers, such as CRP. A slight increase in CRP levels was indeed observed after bonding, but there was no significant difference in salivary CRP levels before and after exposure to fixed orthodontic appliances. MacLaine et al.²⁹ examined the levels of systemic inflammatory markers such as plasma CRP in patients before and during orthodontic treatment. They concluded that exposure to fixed orthodontic appliances was not associated with significant changes in serum CRP levels. In another study,³⁰ it was found that treatment with fixed orthodontic appliances was associated with increased levels of serum CRP, both on the first day and 3 months after the beginning of treatment. In the current study, two time points were chosen (1 hour after bonding and 4–6 weeks later). By this choice, it is possible that the crucial time point when inflammatory events and stress occur was missed. To answer this question, a future time kinetic study will be essential.

Using a strip-based test to assess the salivary pH, a significant decrease in the pH of subjects immediately after the bonding of fixed orthodontic appliances was observed. This may be accounted for by the exposure to 37% phosphoric acid used for enamel etching prior to bonding of the orthodontic appliances. If this is the cause, it would be expected that the pH would return to normal as time elapsed. Indeed, after 4–6 weeks, the pH values returned to normal. Several investigators^{19–21,23} found a significant increase in salivary pH a few weeks after exposure to fixed orthodontic appliances, while others²² found no significant change. Previous studies in healthy individuals^{31–33} found the mean values of salivary pH to be 6.82, 6.78, and 6.8, respectively, similar to the current findings.

Employing a strip-based test, there was no significant difference in the presence of blood in saliva of subjects before and after exposure to fixed orthodontic appliances. Various studies have found that orthodontic treatment was associated with increased gingival bleeding.^{16,34} In this study, a significant increase in the presence of blood in saliva of orthodontic patients was not detected. This could be accounted for by the low specificity of the method used. It could also be explained by the fact that gingival inflammation is local and probing is necessary to induce bleeding into the oral cavity.

Salivary protein presence was assessed by a strip-based test, which is especially sensitive in the presence of albumin. Exposure to fixed orthodontic appliances had no significant effect on salivary proteins. Indeed, a recent study on blood samples from patients before and during orthodontic treatment found no significant changes in the levels of albumin after 3 months of treatment,³⁰ in agreement with the current findings.

CONCLUSIONS

- Exposure to fixed orthodontic appliances does not seem to have a significant effect on salivary OSA, cortisol, CRP, proteins, or blood, with the exception of a significant but transient pH decrease after bonding.
- More studies are needed to further evaluate the effect of exposure to fixed orthodontic appliances on salivary parameters in different populations and at different time points.

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