

Evaluation of salivary total oxidant-antioxidant status and DNA damage of children undergoing fixed orthodontic therapy

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ABSTRACT

Objective: To determine the total oxidant status (TOS), total antioxidant status (TAS), and the 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels and their interrelationship in the saliva of children undergoing fixed orthodontic therapy.

Materials and Methods: Thirty children were randomly divided into three groups. The attachments were bonded to all of the teeth using three different orthodontic composites: Transbond XT, Kurasper F, and GrenGloo. The salivary levels of TOS, TAS, and 8-OHdG were determined three times, as follows: before treatment (T₁) and at 1 month (T₂) and 3 months (T₃) following appliance placement. All data were statistically analyzed.

Results: There were no significant differences in TOS, TAS, and 8-OHdG within the same time periods among the three different orthodontic composites ($P > .05$). TAS in all composite groups decreased over time. These decreases were found to be significant for Kurasper F and GrenGloo at the T₁-T₃ and T₂-T₃ time periods ($P < .05$). In all composite groups 8-OHdG decreased between T₁ and T₂ ($P < .05$). However, 8-OHdG in all composite groups increased from T₂ to T₃. These differences in 8-OHdG were significant in Kurasper F and GrenGloo ($P < .05$).

Conclusions: Fixed orthodontic appliances bonded with the tested composites did not increase the cytotoxicity markers in saliva. (*Angle Orthod.* 2015;85:239-244.)

KEY WORDS: Total oxidant status; Total antioxidant status; 8-Hydroxy-2'-deoxyguanosine

INTRODUCTION

Orthodontic composites are often used by pediatric dentists and orthodontists for the bracket-banding process in children with both primary and permanent dentition. However, some components of the orthodontic composites may be released into the oral

environment and saliva during fixed-appliance treatment, and even following polymerization.^{1,2} These components released from orthodontic composites are triethylene-glycoldimethacrylate (TEGDMA), urethane dimethacrylate (UDMA), 2-hydroxyethylmethacrylate (HEMA), bisphenol A, bisphenol A-diglycidyl dimethacrylate (Bis-GMA), and methyl methacrylate. The release of these components and their diffusion may cause various adverse effects in the organism, such as allergic reactions, systemic toxicity, cytotoxicity, mutagenicity, and carcinogenicity.³⁻⁵

Although there has been satisfactory development of orthodontic composite materials, the biocompatibility of these materials is usually disregarded in dental practice. Evaluating the cytotoxicity, genotoxicity, and biocompatibility of orthodontic composites and luting cements is as important as considering the physiological or mechanical properties of these materials. However, there are limited studies relating to the cytotoxic effects of orthodontic adhesives.⁶⁻⁹

Antioxidants protect against the potentially harmful effects of processes or reactions that cause excessive oxidation. The total oxidant status (TOS) reflects the present oxidative status. The total antioxidant status (TAS) provides information about the antioxidant

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Table 1. Orthodontic Composite Used in This Study^a

Orthodontic Composite	Application Time, s	Material Content	Manufacturer
Transbond XT Light Cure	20	Bis-GMA, Bis-EMA, TEGDMA silane-treated quartz, silane-treated silica	3M Unitek Ortho Prod, Monrovia, CA, USA.
Kurasper F Light Cure	40	Bis-GMA, TEGDMA, silanated barium glass filler, colloidal silica, DL-camphorquinone, catalysts and accelerators	Kuraray Europe GmbH, Frankfurt, Germany
GrenGloo Light Cure	20	Uncured methacrylate ester monomers, inert mineral fillers, fumed silica, activators and preservatives	Ormco Corporation, Glendora, CA, USA.

^a%38 Phosphoric acid applied for 30 seconds; light-curing time was carried out per manufacturer's instructions.

^a Bis-GMA indicates bisphenol A diglycidylmethacrylate; Bis-EMA, bisphenol A Bis (2-hydroxyethyl ether) dimethacrylate; and TEGDMA, triethylene glycoldimethacrylate decamethylene dimethacrylate.

capacity of the organism. There are few studies on the antioxidant defense systems of saliva and their relationship to oral disease in children.^{10,11}

Oxidative damage to DNA can be detected by chemical, physical, and enzymatic methods. The levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) have been used to evaluate DNA damage. It is possible to prove cytotoxicity via oxidative stress by detecting 8-OHdG. Therefore, it has often been used as a biomarker for oxidative damage.¹²

In the literature, there are some reports^{6-9,13} on the genotoxic and cytotoxic effects of different orthodontic materials. However, the TOS, TAS, and 8-OHdG levels in the saliva of children undergoing fixed orthodontic therapy have not yet been studied. The aim of the present study was to determine the relationship between the salivary levels of TOS, TAS, and 8-OHdG in children undergoing fixed orthodontic therapy with three different orthodontic composites over a 3-month evaluation period.

MATERIALS AND METHODS

The healthy children, aged between 11 and 17 years (mean age, 13.72 ± 1.7 years) were selected from consecutive patients referring for orthodontic treatment to the Department of Orthodontics at Inonu University, who fulfilled the following inclusion criteria: they were nonsmokers, nondrinkers, were not taking medications, and were not exposed to systemic infections; they had good oral hygiene; they had no decay, fillings, or gingival inflammation; and they had not been using oral-antiseptic solutions or topical fluoride during tooth development.

Their informed consent was obtained, and the study was approved by the Faculty of Medicine Ethic Committee, Inonu University (2011-169). All of the patients underwent lateral and frontal cephalometric radiography and panoramic dental radiography. Three months after the X-ray exposure, orthodontic treatment was initiated.

A total of 30 children who fulfilled inclusion criteria were selected for this study. A power analysis conducted during the planning of the study showed

that a sample size of 10 participants would provide sufficient statistical power to discriminate changes in the salivary variables under study. Power analysis was performed with MINITAB version 14.1 software (Minitab Inc, State College, Pa). The selected patients were randomly divided into three equal groups according to the orthodontic composite used in this study (n = 10, alpha level of 0.05, and Power of 0.80). The orthodontic composites used in this study are shown in Table 1. The three orthodontic composites were randomly assigned in a consecutive manner. Transbond XT Light Cure (3M Unitek Ortho Prod, Monrovia, Calif) was applied to the first child who participated in the study. Kurasper F Light Cure (Kuraray Europe GmbH, Frankfurt, Germany) was applied to the second child who participated in the study, and GrenGloo Light Cure (Ormco Corporation, Glendora, Calif) was applied to the third child who participated in the study. This consecutive assignment of orthodontic composite was then repeated for all subsequent participating children. For standardization purposes, Equilibrium 2 brackets and double-direct molar tubes (Dentaurum, Ispringen, Germany) were bonded with three different orthodontic, light-cure composites (according to the manufacturer's instructions) to all teeth in both arches, including the second molars if they had erupted. In each group, 0.012-, 0.014-, and 0.016-inch Rematitan ideal, round, nickel-titanium archwires (Dentaurum) were applied with 0.010 inch Remanium preformed, stainless-steel ligature wires (Dentaurum) at the 2-month interval.

Saliva samples were collected three times from each patient. The first samples were collected before the appliance placement and were considered as the controls (T₁). One month after the appliance placement, the same investigator took the second set of samples (T₂). The final records (T₃) were obtained 3 months after appliance placement.

Saliva Collection

Unstimulated saliva samples were collected between 9 AM and noon. Children were instructed not to eat or drink anything for at least 1 hour before

Table 2. Distribution of Total Oxidant Status (TOS) According to Orthodontic Composite Resin^a

Orthodontic Composite Groups	TOS, $\mu\text{mol H}_2\text{O}_2$ Equiv/L					
	T ₁ Median (Min–Max)	T ₂ Median (Min–Max)	T ₃ Median (Min–Max)	P		
				T ₁ –T ₂	T ₁ –T ₃	T ₂ –T ₃
Transbond XT	14.36 (4.5–34.3) A	19.78 (3.5–30.6) A	24.25 (3.0–72.3) A	.263	.327	.484
Kurasper F	23.33 (12.2–47.2) A	16.80 (5.5–88.1) A	23.10 (6.6–55.5) A	.779	.674	.674
GrenGloo	21.08 (4.6–33.0) A	22.15 (4.5–59.3) A	15.49 (2.2–60.0) A	.463	.917	.345

^a The nonparametric Wilcoxon matched-pairs test was significant at $P < .05$ when baseline value (T₁) was compared with the values 1 (T₂) and 3 (T₃) months after appliance placement. Means with the same letter were not significantly different ($P > .05$).

collecting the saliva sample and to brush their teeth once in the morning on the day of salivary collection. Children sat in a dental chair at leisure, slightly bent forward, and were instructed to collect the unstimulated saliva into a sterile plastic cup over the course of a 5-minute period. The collected saliva samples were stored at -80°C until biochemical analysis was carried out. The assays for TOS, TAS, and 8-OHdG in saliva were performed in triplicate, and the arithmetic means were calculated.

Assays for TOS and TAS

The TOS in the saliva samples was measured using a novel colorimetric and automated-measurement method for TOS developed by Erel.¹⁴ The assay is calibrated with hydrogen peroxide, and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter ($\mu\text{mol H}_2\text{O}_2$ Equiv/L).

The TAS in the saliva samples was measured using a novel colorimetric and automated direct-measurement method for TAS developed by Erel.¹⁵ In this assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) is incubated with metmyoglobin and hydrogen peroxide to produce ABTS⁺. Antioxidants present in the saliva cause a reduction in the absorption that is proportional to their concentration. The total antioxidant capacity value of the tested saliva is expressed as an equivalent of the millimolar concentration of Trolox solution ($\mu\text{mol Trolox Equiv/L}$). The inter- and intra-assay coefficients of variation were 14.62 and 4.85 for TOS and 14.15 and 3.36 for TAS, respectively.

Assay for 8-OHdG Levels

The values of 8-OHdG were determined with test kits involving the enzyme-linked immunosorbent assay (ELISA) method (CAYMAN ELISA Kit 8-hydroxy-2'-deoxyguanosine, Ann Arbor, MI, USA). The sensitivity of Assay Designs' DNA Damage ELISA kit was determined to be 0.59 ng/mL. The inter- and intra-assay coefficients of variation were 4.1 and 5.2, respectively.

Statistical Analysis

The data were analyzed using SPSS for Windows, version 16.0 (SPSS Inc, Chicago, Ill). Descriptive statistics (mean, median, standard deviation, and minimum and maximum values) were calculated. The nonparametric Wilcoxon matched-pairs test was used to analyze the differences in TOS, TAS, and 8-OHdG levels in the saliva at the different time periods within the same orthodontic composite group. The statistical significance of differences in TOS, TAS, and 8-OHdG levels in the saliva at the different time periods within the same orthodontic composite group was accepted at the $\alpha < .05$ level.

The Kruskal–Wallis test was used to analyze the differences in the TOS, TAS, and 8-OHdG salivary levels for the same time period among the three different orthodontic composite groups. The statistical significance of differences in TOS, TAS, and 8-OHdG salivary levels for the same time period among the three different orthodontic composite groups was accepted at the $\alpha < .05$ level.

Table 3. Distribution of Total Antioxidant Status (TAS) According to Orthodontic Composite Resin^a

Orthodontic Composite Groups	TAS, $\mu\text{mol Trolox Equiv/L}$					
	T ₁ Median (Min–Max)	T ₂ Median (Min–Max)	T ₃ Median (Min–Max)	P		
				T ₁ –T ₂	T ₁ –T ₃	T ₂ –T ₃
Transbond XT	0.41 (0.08–0.58) A	0.29 (0.08–0.70) A	0.20 (0.04–0.50) A	.674	.128	.401
Kurasper F	0.40 (0.04–0.72) A	0.21 (0.08–0.64) A	0.09 (0.02–0.46) B	.092	.036	.018
GrenGloo	0.37 (0.28–0.68) A	0.18 (0.04–0.40) A	0.07 (0.02–0.20) B	.128	.028	.025

^a The nonparametric Wilcoxon matched-pairs test was significant at $P < .05$ when baseline value (T₁) was compared with the values 1 (T₂) and 3 (T₃) months after appliance placement. Means with the same letter were not significantly different ($P > .05$).

Table 4. Distribution of 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) According to Orthodontic Composite Resin^a

Orthodontic Composite Groups	8-OHdG, ng/mL			<i>P</i>		
	T ₁ Median (Min–Max)	T ₂ Median (Min–Max)	T ₃ Median (Min–Max)	T ₁ –T ₂	T ₁ –T ₃	T ₂ –T ₃
	Transbond XT	81.11 (31.3–219.4) ^A	31.01 (20.8–90.1) ^B	39.1 (10.3–76.3) ^B	.012	.036
Kurasper F	75.02 (19.0–195.6) ^A	32.39 (20.6–117.8) ^B	108.88 (24.1–172.5) ^A	.036	.674	.036
GrenGloo	39.44 (30.3–50.2) ^A	23.15 (10.3–27.2) ^B	36.95 (10.3–70.0) ^A	.028	.686	.049

^a The nonparametric Wilcoxon matched-pairs test was significant at $P < .05$ when baseline value (T₁) was compared with the values 1 (T₂) and 3 (T₃) months after appliance placement. Means with the same letter were not significantly different ($P > .05$).

RESULTS

Statistically significant differences were not found in the TOS, TAS, and 8-OHdG levels in the saliva for the same time periods from among the three different orthodontic composites ($P > .05$) (Tables 2–4). The levels of TAS in all composite groups decreased over time (Table 3). These decreases were found to be statistically significant for Kurasper F and GrenGloo at the T₁–T₃ and T₂–T₃ time points ($P < .05$). The levels of 8-OHdG in all composite groups decreased between T₁ and T₂ ($P < .05$) (Table 4). However, the levels of 8-OHdG in all composite groups increased from T₂ to T₃. These differences in the levels of 8-OHdG in the saliva were statistically significant in the Kurasper F- and GrenGloo-applied groups ($P < .05$).

The effect on TOS, TAS, and 8-OHdG levels in the saliva of the three different orthodontic composites used in this study was found to be similar during the 3-month evaluation period.

DISCUSSION

The appliances in fixed orthodontic treatments are fabricated from different alloys. These alloys include nickel, cobalt, and chromium. These metallic ions and monomers released from orthodontic composites have harmful effects on the adjacent oral tissues. In addition, it has been reported^{16,17} that the mucosa of the mouth may absorb residual monomers and that these monomers may be taken into the digestive system by means of the saliva. Therefore, in the present study we aimed to evaluate the long-term effects of metallic ions and monomers released from orthodontic composites on TOS, TAS, and 8-OHdG levels in the saliva.

Antioxidants are an important part of our diet, and these systems, together with intracellular antioxidants and enzymatic systems, impede inflammation, infections, and tumor formation.¹⁸ Some types of inflammation, especially periodontal disease, have been associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity.¹⁹

Oxidative stress has been defined as a disturbance in the oxidant-antioxidant balance, resulting in poten-

tial cell damage. It is known that oxidative stress plays a role in the pathogenesis of cancer and many inflammatory diseases, such as diabetes mellitus, atherosclerosis, hypertension, and obesity.²⁰ Antioxidant defense systems have been found to prevent the formation of free radicals and to reduce cell damage. Sculley and Langley-Evans²¹ stated that women had significantly lower TAS levels than men, regardless of their periodontal health. In addition, the authors reported that salivary TAS increased with increasing caries activity.^{11,22–26} Tóthová et al.²⁶ found that salivary antioxidant status in children was related to oral hygiene and periodontal status. To assess the results with accuracy, all of the patients who were selected for this evaluation had no fillings, no caries, and no periodontal disease. We found that the levels of TAS in all composite groups decreased over time. These decreases were statistically significant for Kurasper F and GrenGloo at the T₁–T₃ and T₂–T₃ time points ($P < .05$). These decreases may be related to a lack of fillings, lack of caries, good oral hygiene, and fluoride release from the orthodontic composites.

8-OHdG is a biomarker that indicates oxidative damage in a number of disorders, including chronic inflammatory diseases.¹² Salivary 8-OHdG levels have been intensively studied in children, including in patients with periodontitis.²⁷ Takane et al.²⁷ reported that salivary 8-OHdG levels appeared to reflect the status of periodontal health. In addition, the latest data showed that the antioxidant capacity decreased and that certain oxidative stress biomarkers increased in periodontitis patients.^{28–30} We found that the levels of 8-OHdG in all composite groups decreased between T₁ and T₂ ($P < .05$). However, the levels of 8-OHdG in all composite groups increased from T₂ to T₃. These differences in the levels of 8-OHdG were statistically significant in the Kurasper F- and GrenGloo-applied groups ($P < .05$). These differences may be related to composite content, the release of monomers, and the release of fluoride.

In this study, we found that orthodontic fixed attachments that were bonded with three different orthodontic composites showed no cytotoxic effects in children during the 3-month evaluation period. This

finding may result from the influence of the adhesive content and the properties of metallic appliances, either separately or jointly. Transbond XT and Kurasper F contain Bis-GMA and TEGDMA as a main component. GrenGloo contains uncured methacrylate ester monomers. In the literature, Bis-GMA, TEGDMA, UDMA, and HEMA were tested as resin components. Kleinsasser et al.³¹ stated that TEGDMA, UDMA, and HEMA induced a significant elevation in DNA migration in the Comet assay and that this was a possible sign of genotoxic effects in human salivary glands and lymphocytes. In our in vivo study we applied the same metallic devices and three different commercial compound-type bonding adhesives. This could be a factor leading to the decrease in the release of monomers.

A combination of monomers is generally used in orthodontic composites. Tested orthodontic composites include mostly similar resin matrices. However, Transbond XT also contains Bis-EMA. In addition, a Bis-EMA monomer showed a cytotoxic effect analogous to that of TEGDMA.³² Lee et al.⁶ stated that all experimental monomers exhibited a dose-dependent cytotoxic effect, and the ranking of the cytotoxicity was GMA > TEGDMA > HEMA. The increased DNA damage in cementation with Kurasper F Light Cure has been mainly attributed to the release of the monomers TEGDMA and Bis-GMA, which are added to this chemical composition.

This study does have some limitations. One limitation is that all subjects were recruited from among children at the University Hospital. This may limit the ability to extrapolate the present findings to the general population. The second limitation is that only patients with good oral hygiene were included in this study. Caries and gingival inflammation may result in a change in terms of the salivary variables.

CONCLUSIONS

- The levels of TAS in all composite groups decreased over time.
- Fixed orthodontic appliances applied using three tested orthodontic composites have no cytotoxic effects on children. Therefore, tested composites can be used safely by pediatric dentists or orthodontists.

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