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Araştırma/ Research Article

TRACE ELEMENT LEVELS IN SALIVA IN SUBJECTS WITH COMPOSITE FILLING

KOMPOZİT DOLGU YAPILAN BİREYLERDE TÜKÜRÜK ESER ELEMENT SEVİYESİ

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ABSTRACT
Aim: Trace elements are agents that regulate various biological pathways. Copper and zinc are essential components of the antioxidant enzyme system. The aim of this study was to determine iron, copper, and zinc levels in saliva after application of composite resin restoration.
Materials and Methods: Twenty cases of class I caries cavity (M:F ratio 8:12; age 18-25 years) were selected for the present study. Following cavity preparation, the restorative material (Filtek Z 250) was applied to the cavities in line with the manufacturer's instructions. Unstimulated whole saliva samples were collected from before, and one hour, one day, seven days, and 30 days after restoration. Trace element saliva contents (zinc, copper, and iron) were analyzed using an atomic absorption spectrophotometer (AAS) (AAnalyst 800, Perkin Elmer, USA) with the electrothermal graphite oven technique. Repeated measures analysis of variance was used to evaluate trace element levels over time (α=0.05).
Results: Copper, zinc, and iron levels in saliva decreased from 0.189±0.138 µg/ml to 0.123±0.031 µg/ml, from 0.067±0.012 µg/ml to 0.060±0.013 µg/ml, and from 0.162±0.049 µg/ml to 0.126±0.032 µg/ml, respectively, at the end of 30 days. However, these changes were not statistically significant (p>0.05).
Conclusions: Composite resin used in the present study did not affect trace element levels in saliva.
Keywords: composite resin, copper, iron, saliva, zinc

ÖZ
Amaç: Eser elementler, çeşitli biyolojik yolları düzenleyen maddelerdir. Bakır ve çinko antioksidan enzim sisteminin temel bileşenleridir. Bu çalışmanın amacı, kompozit rezin restorasyonu uygulandıktan sonra tükürükteki demir, bakır ve çinko seviyelerini belirlemektir.
Materyal ve metot: Çalışmaya sınıf I çürük kavitesine sahip 20 adet birey dahil edildi. (E:K oranı 8:12; yaş 18-25 yıl). Kavite pereperasyonundan sonra restoratif materyal (Filtek Z 250) üretici firma talimatlarına göre uygulandı. Uyarılmamış tükürük örnekleri restorasyondan önce, restorasyondan 1 saat, 1 gün, 7 gün ve 30 gün sonra toplandı. Tükürükteki eser element miktarı elektrotermal grafit fırın tekniği ile atomik absorbsiyon spektrofotometresi (AAS) (AAnalyst 800, Perkin Elmer, USA) kullanılarak ölçüldü. Zamanla eser element miktarındaki değişim, tekrarlı ölçümler varyans analizi kullanılarak değerlendirildi (α=0.05).
Bulgular: Tükürükteki bakır, çinko ve demir seviyeleri 30 gün sonunda sırasıyla 0.189±0.138 µg/ml'den 0.123±0.031 µg/ml'e , 0.067±0.012 µg/ml' den 0.060±0.013 µg/ml' ye 0.162±0.049 µg/ml'den 0.126±0.032 µg/ml'ye düşüş göstermektedir. Ancak bu değişiklikler istatistiksel olarak anlamlı değildir (p>0.05).
Sonuç: Bu çalışmada kullanılan kompozit rezin tükürük eser element seviyesini etkilememiştir.
Anahtar kelimeler: bakır, çinko, demir, kompozit rezin, tükürük

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INTRODUCTION

Composite resin materials are restorative solutions widely employed by dentists due to the adoption of conservative approaches and increasing esthetic demands by patients.[1] The performance of a composite resin depends on its composition, the amount and type of filler, the filler matrix bonding, and degrees of polymerization.[2] The enzymatic activities of saliva and wear result in susceptibility to biodegradation, and oral tissues are exposed to released monomers for extended periods of time.[3]

Incomplete resin polymerization may result in unreacted monomers being released from the restorative materials into the aqueous environment of oral cavity and may cause adverse biological effects.[4] Moreover, an oxygen-inhibited layer is formed at the surface of the resin and causes an increase in free monomer levels. Oxygen causes the formation of unreactive proxy radicals.[5] Additionally, released monomers result in free radicals which are fundamental to any biochemical process and represent an essential component of aerobic life and human metabolism. The imbalance between antioxidants and free radicals plays an important role in the development of reactive oxygen species (ROS), oxidative stress, and oxidative damage.[6, 7]

Trace elements are agents that regulate various biological pathways and compose 0.5% of saliva, together with electrolytes and organic molecules.[8] Copper, zinc, selenium, and manganese are essential components of the antioxidant enzyme system. Trace elements and several antioxidants function together in the human body. Zinc and copper act as cofactors in regulating the functions of superoxide dismutase (SOD), an essential antioxidant enzyme for defense against free radicals.[9] Zinc plays essential roles in the regulation of cell growth, differentiation and division. High copper levels have been observed to exhibit a protective effect against chemical induction.[10] Iron is associated with peroxidases, cariostatic enzymes.[11] Previous studies have shown that iron-sucrose reduces the prevalence of dental caries.[12] It also participates in energy-producing reactions in all cells. Iron activates energy-producing oxidizing enzymes and is important for DNA, RNA antibody and collagen synthesis.[10] It is also responsible for the production of nitric oxide by the redox reaction between nitrite and iron.[13] Nitric oxide exhibits deleterious effects on DNA, cellular proteins and lipids, and can lead to tissue injury, cell death and organ failure. Researchers have studied the role of saliva trace elements in patients with dental caries, and malignant oral lesions.[9,10,12] However, saliva trace elements levels has not been evaluated in composite filled individuals. The aim of this study was to evaluate iron, copper, and zinc levels in saliva after application of composite resin restoration. The hypotheses tested in this study was that composite filling application may not affect zinc, copper, and iron trace elements levels in unstimulated saliva.

MATERIAL AND METHODS

Study design and case selection

Twenty individuals with class I caries cavity (M: F ratio 8:12; age 18-25 years) were included to the study. Ethical approval for the study was granted by the Ethical Committee of the Ataturk University, Faculty of Dentistry, and the research was conducted in accordance with the ethical principles of the Declaration of Helsinki. G*Power 3.1.9.4 software (Heinrich-Heine Dusseldorf University, Dusseldorf, Germany) was used to determine the sample size based on using the following parameters: 85% power, 0.58 effect size, and α error at 0.05. A minimum sample size of 20 participants was assessed to be appropriate. Informed consent forms were obtained from all participants. The inclusion criteria were not currently using any medications, a negative medical history, no use of alcohol or smoking, and absence of periodontal problems. Following cavity preparation, the microhybride restorative material (Filtek Z 250, 3M ESPE Dental Products, St Paul, MN, USA), commonly used in our clinic, was applied to the cavities in line with the manufacturer’s instructions. Materials used for dental restorations are shown in Table 1. Fifty-four single-surface composite restorations were applied to 20 individuals by the same operator. The composite material was given an anatomical form by placing it into cavities not exceeding 2 mm in depth in one piece (the bulk method) followed by polymerization using a light source (Elipar Freelight II, 3M-ESPE Dental Products). The wavelength of the light source was 430-480 nm, and the light intensity was approximately 1200 mW/cm^2. During the polymerization process, the tip of the light source was kept as close to the restoration as possible. The intensity of the light device was measured using a radiometer (Hilux Ultra Plus Curing Units; Benlioglu Dental, Ankara, Turkey). Finishing and polishing procedures were completed using discs (Sof-Lex; 3M ESPE Dental Products).

Table 1. Materials used for dental restorations.

Material	Manufacturer	Content
Filtek Z250	3M ESPE Dental Products, St Paul, MN, USA	TEGDMA 1-5%, Bis-GMA 1-5%, ethoxylated bisphenol-A dimethacrylate (Bis-EMA) 5-10%, and UDMA 5-10%.
Single Bond adhesive	3M ESPE Dental Products	BisGMA 10-30%, HEMA 5-25%, and dimethacrylates 7-28%.
Scotchbond Etchant gel	3M ESPE Dental Products	38% phosphoric acid

Collection of saliva samples

Unstimulated whole saliva samples were collected between 9 and 11 AM by a single operator. During the procedure, participants were seated in a relaxed position with the head bent forward to allow saliva to accumulate in the anterior oral cavity. The participants then swallowed, after which saliva was collected into tubes for 15 minutes. The participants had been instructed not to eat or to drink (apart from water) for two hours prior to saliva collection. Samples were collected before the restoration, and one hour, one day, seven days, and 30 days after the restora-tion. Follow-up periods were determined according to the monomer release times stated in the literature.[14] Samples were stored at -80^{0}C until analysis.

Analysis of trace element levels

The trace element content of saliva (zinc, copper, and iron) was analyzed using an atomic absorption spectrophotometer (AAS) (AAnalyst 800, Perkin Elmer, USA) with the electrothermal graphite oven technique. Briefly, 0.5 mL of concentrated nitric acid (HNO_3) was added to the saliva sample to prevent any enzymatic changes and microbial growth. The saliva was then centrifuged at 4000 rpm for 10 min to remove mucin clots and food debris that might block the AAS capillary tube.[15] The wavelength, light source, and matrix modifier used for the measured elements are shown in Table 2. A calibration curve was produced to determine the concentration of each element using the results obtained from the working standard solutions containing different known concentrations of the element and the same reagents as those used for the saliva samples.

Table 2. The wavelength, light source, and matrix modifier used for the measured elements.

Elements	Wavelength (nm)	Matrix modifier (Merck)	Light source
Cu	324.8	5 μg Pd + 3 μg $Mg(NO_3)_2$	HDL Hollow cathode lamp
Fe	248.3	15 μg $Mg(NO_3)_2$	
Zn	213.9	5 μg $Mg(NO_3)_2$	

Statistical analysis

Statistical analysis was performed on SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) software. The repeated measures analysis of variance test was used in the assessment of trace element levels over time (α=0.05).

RESULTS

Changes in levels of salivary trace elements and the statistical comparisons results are given in the figure 1. Repeated measures of variance analysis for each element showed that trace element levels in saliva decreased over time after composite filling application. Copper, zinc, and iron levels in saliva decreased from 0.189±0.138 μg/ml to 0.123±0.031 μg/ml, from 0.067±0.012 μg/ml to 0.060±0.013 μg/ml, and from 0.162±0.049 μg/ml to 0.126±0.032 μg/ml, respectively, at the end of 30 days. However, these changes were not statistically significant ($p>0.05$).

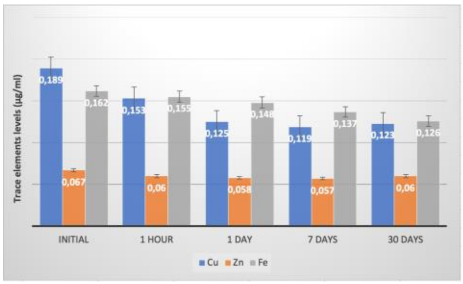

Figure 1. Mean salivary trace element levels after composite filling.

DISCUSSION

The study findings demonstrated that composite filling application caused a slight, statistically insignificant decrease in trace element levels in saliva. Based on the results, the study hypothesis was confirmed.

Saliva is a complex and unique secretion, with numerous functions in the oral cavity, such as preventing caries, lubrication and protection of oral soft tissues, and modulating oral microbial ecosystems.[8, 16] Mixed saliva collection is preferred in the diagnosis of oral and systemic diseases due to its non-invasive nature and simplicity. Levels of hormones, proteins, antibodies, and other molecules in saliva are frequently measured to determine health and disease status.[17] Changes in saliva composition depend on a range of physiological, pathological and environmental factors.[8] Experimental studies have confirmed that monomers released from dental composite within the 28-days and maximum monomer concentration observed within the first seven days.[14] Released monomers affect the redox balance and material toxicity in association with enhanced ROS production.[18-20] Biological fluids possess protective antioxidant mechanisms to prevent the production of free radicals and to repair oxidative damage. Composite resin materials activate the antioxidant system in saliva.[21] Trace elements are the main components of the antioxidant enzyme system. Various previous studies have investigated trace element levels in patients with dental caries, and malignant and premalignant oral lesions.[22, 23] However, to the best of our knowledge, this is the first study to assess copper, zinc, and iron levels in after composite filling.

Zinc acts as a cofactor for the enzyme Cu-Zn SOD, part of the primary antioxidant system. Some research has reported lower serum zinc levels in patients with premalignant disorders such as oral leukoplakia, possibly due to consumption of zinc in the counter reaction to oxidants.[23] In the present study, salivary zinc levels of saliva decreased by approximately 10% from baseline at the end of the 30th day. However, this was not statistically significant. A reduction in salivary zinc levels may be related to changing SOD activity. Gul et al.[24] reported that monomers released after composite filling application may lead to oxidative stress and alter salivary antioxidant enzyme activity. Ramezani et al.[25] reported significantly higher salivary total antioxidant capacity (TAC) levels in children with dental composite restorations compared to dental amalgam and caries-free samples. The authors then suggested that the increase in TAC levels may be associated with degradation products of dental composite. In a study performed by Guler et al.[26] showed decreased TAC level over time in children with orthodontic appliances bonded with orthodontic composite. Yildiz et al.[27] reported that composite material in rabbit connective tissue implants increased SOD activity on both days 1 and 7.

Based on our results, mean salivary copper levels decreased by approximately one hour after composite filling application. At the end of the 30th day, the decrease was approximately 35%. This change was also not statistically significant. Recent research has reported higher salivary copper level in caries groups than in a caries-free group and attributed this to the demineralization by caries of the tooth structure.[28] However, copper is also a component of SOD, which regulates the intercellular concentration of superoxide anion by converting it to hydrogen peroxide. Huang et al.[29] reported that cooper, zinc, and manganese ions correlate with salivary SOD levels in periodontal diseases, indicating that these redox-active micronutrients play a crucial role in regulating oxidative stress status.

Salivary iron levels were 0.126 µg/ml at the end of 30 days. This decrease of 22% compared to baseline was not statistically significant. Iron is an oxygen-carrying pigment of red blood cells and also participates in energy-producing reactions in all the cells. Iron activates energy producing oxidizing enzymes and is crucial to antioxidant processes, since a change in Fe concentrations may result in ROS formation.[10, 30] Watanabe et al.[31] compared the effects of composite resin and metal restoration application on salivary iron levels and reported that iron concentrations were significantly affected by composite resin restoration. They noted that iron was eluted from teeth with composite restoration due to residual ferric chloride originating from the etching agent. The interactions and associations between salivary iron levels and dental decay have been investigated for several years. Consistent with the present study, iron has not been shown to exhibit any consistent association with dental caries.[32, 33]

The small number of participants and using one type of composite resin are the limitations of the study. Hence, more research need to be conducted on the effect of different type of resin composite in larger number of participants.

CONCLUSIONS

Within the limitations of this study, salivary zinc, copper, and iron levels did not change significantly after microhybride composite filling application. Further studies are now needed to clarify the relationship between trace elements in saliva and oxidative stress parameters in subjects with composite filling.

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Conflict of Interest:
The authors declare that they have no conflict of interest.

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