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Salivary bisphenol A levels and their association with composite resin restoration

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HIGHLIGHTS

- The level of BPA in saliva collected 5 min after filling procedure was 3.64 μg/L.
- The level of BPA in saliva 7 d after filling procedure was 0.59 μg/L, which was lower compared to level 5 min after procedure.
- On the basis of the EFSA criterion, level of BPA after filling procedure was assessed as safe and found not harmful to health.

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ABSTRACT

Composite resin has been increasingly used in an effort to remove minimal amount of tooth structure and are used for restoring not just carious cavities but also cervical abrasion. To synthesize composite resin, bisphenol A (BPA) is used. The aim of the study was to measure the changes in salivary BPA level related with composite resin restoration. ELISA was used to examine the BPA levels in the saliva collected from 30 volunteers whose teeth were filled with composite resin. Salivary samples were collected immediately before filling and 5 min and 7 d after filling. Wilcoxon signed-ranks test and linear regression were performed to test the significant differences of the changes in BPA levels in saliva. Before a new composite resin filling, there was no significant difference between with and without existing filling of composite resin and BPA level in the saliva was not correlated to the number of filled surfaces with composite resin. However, BPA level in the saliva increased to average 3.64 μg/L from average 0.15 μg/L after filling 5 min. BPA level increased in proportion with the number of filled surfaces. BPA level decreased to average 0.59 after filling 7 d. However it was higher than the BPA level before a new composite resin filling. Considering 50 μg/kg/day as the Tolerable Daily Intake of BPA suggested by European Food Safety Authority, the amount of BPA eluted in saliva after the composite resin filling is considered a safe level that is not a hazard to health at all.

1. Introduction

Mastication is a fundamental function to sustain survival behavior of eating and drinking. It is difficult to conduct everyday activities if teeth are lost (Kim et al., 2009a, 2009b). Dental caries, also known as tooth decay, cavities, or caries, is a breakdown of teeth due to activities of bacteria (Silk, 2014). Dental caries and periodontitis have been reported as leading causes of tooth loss both in and outside Korea (Richards et al., 2005; Aida et al., 2006; Ro et al., 1998; Lee et al., 2002). To prevent tooth loss, the primary strategy is to prevent dental caries and periodontitis, but if the disease is already present, secondary prevention in the form of

Abbreviations: BPA, Bisphenol A; Bis-GMA, Bisphenol A-glycidyl dimethacrylate; Bis-DMA, Bisphenol A-dimethacrylate; BADGE, Bisphenol A-diglycidyl ether; Bis-EMA, Bisphenol A-ethoxylate dimethacrylate; TEGDMA, Triethylene glycol dimethacrylate; UDMA, Urethane dimethacrylate; MFDS, The Korean Ministry of Food and Drug Safety; TDI, Tolerable daily intake; EFSA, European Food Safety Authority.

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early treatment is important to prolong the lifespan of teeth (Kim et al., 2009a, 2009b).

Dental filling materials frequently used for restoring teeth with dental caries include amalgam, composite resin, glass ionomer cement, and casting alloy (inlay and onlay) (Kim et al., 2012a, 2012b). As carious cavities are holes at the surface of the tooth by the bacteria acids, amalgam had been the first choice for filling carious cavities because of its relatively low price for a long time (Bayne et al., 2013). However its color differs from the natural color of teeth. Recently, composite resin has been increasingly used in an effort to remove minimal amount of tooth structure to retain the filling material (Eley, 1997; Burke et al., 2003; Mjör et al., 1999). Composite resins are used for restoring not just carious cavities but also cervical abrasion cavities (Kim et al., 2012a, 2012b; Al-Agha and Alagha, 2015).

To synthesize plastic products and composite resin, bisphenol A (BPA) is used (Imai and Komabayashi, 2000; Shelby, 2008), which is classified as an endocrine disrupting chemical (Michałowicz, 2014). Bisphenol A (BPA) is a synthetic chemical resin used worldwide in the production of plastic products, notably polycarbonate plastic food-storage containers, some water bottles, bottle tops, and epoxy resin lacquer linings of metal food cans (Fleisch et al., 2010). Other environmental sources, including air, dust and water, also may contribute to total BPA exposure in the general population (Meesters and Schröder, 2002). More than 2 million tons of BPA are currently produced per year, and there is an anticipated 6%–10% annual growth in future demand (Burridge, 2003). BPA works in a manner similar to that of estrogen, and if absorbed into the digestive system or through the skin of a fetus or a baby, it can result in disturbances in the formation of sexual organs and in the development of sexual characteristics during puberty (Veiga-Lopez et al., 2015; Vandenberg et al., 2012, 2010; Vandenberg, 2014). When composite resin fillings or resin-based dental sealants are used to treat tooth decay or cervical abrasion, BPA partially leaches out thus causing a safety concern for general systemic health (Kingman et al., 2012; Kloukos et al., 2013).

In Korea, Han et al. (2012). have investigated the level of BPA leaching into saliva in a group of patients with composite resin fillings or resin-based sealants, and Kang et al. (2011). examined the levels of salivary and urinary BPA in patients undergoing orthodontic treatment before and after they started to wear a composite resin retainer following the corrective phase with braces. However, research has rarely been conducted to compare the levels of BPA leaching into saliva in patients before and after the composite resin filling procedure.

Accordingly, in the current study, we aimed to investigate the change in salivary BPA level after the composite resin filling procedure and examine its effect on systemic health. This was done by collecting saliva from patients undergoing the procedure using the composite resin product for dental restoration.

2. Materials and methods

2.1. Study subjects

The study was conducted on patients who visited a dental clinic for treatment of dental caries or cervical abrasion by filling of these cavities with composite resin, and provided voluntary consent for participation in the study. The inclusion criteria were (1) being systemically healthy, (2) no history of taking antibiotics for the last 6 months, and (3) voluntary participation in the study. The study was approved by Pusan National University Dental Hospital Institutional Review Board (PNUDH-2014-043), and conducted from March to July of 2015 after participants signed a written consent form. There were a total of 30 participants (14 males and 16 females); 13 of them were less than 40 years of age, and 17 were 40 years old or more.

2.2. Saliva collection

Saliva was collected from all the participants before and after the composite resin filling procedure under the same conditions in dental clinic, Busan, Republic of Korea. Right before the procedure was performed, unstimulated saliva was collected for 5 min via a BPA free plastic tube. Then, a rubber dam was held over the maxillary or mandibular molars, and cavities were filled with composite resin (A3, Filtek Z350 XT, 3M ESPE Inc, St Paul, MIN, USA) following the manufacturer's directions for all participants. The filling procedure was conducted to 15 participants per a day for continuous two days. Following the procedure, the mouth was thoroughly rinsed and after waiting for 5 min, the saliva was collected again for 5 min in a separate tube. Seven days after the procedure, the patients revisited the dental clinic for a third collection of unstimulated saliva, which was also collected for 5 min.

2.3. Analytic methods

Collected saliva was immediately cryopreserved in programmable refrigerator at −20 °C and later transported to a laboratory. Before the salivary analysis, we had a sample clean-up procedure to support the efficacy of this procedure to eliminate the matrix effect. An SPE cartridge (Isolute M-M cartridge) was used for sample cleanup for further elimination of impurities from the samples prior to the analysis. We mixed the salivary sample 100 μL, acetic acid buffer solution, pH 5.0 [sodium acetate 1.2 g, acetic acid 1 mL, l-ascorbic acid 0.15 g, EDTA-2Na 0.01 g, distilled water 100 mL] 200 μL, β-glucuronidase (Sigma-Aldrich, from Helix pomatia; 22,000 U/mL) 10 μL and incubated the mixed sample at 37 °C for 18 h [Then, we poured the salivary sample through an Isolute M-M column (Biotage, Sorbent mass; 500 mg, Reservoir volume; 3 mL) preconditioned with methanol (10 mL) and distilled water (6 mL).] After we washed the cartridge with 35% methanol solution (water/methanol = 65:35, 6 mL), eluted the sample with methanol (2.5 mL) and evaporated the solvent with nitrogen. Next, we add 100% methanol to the residue and stirred the mixture with a vortex. Then we terminated the mixing and poured distilled water to adjust the contents at 10% methanol (v/v). Therefore, BPA in the sample dissolved in 10% methanol was applied to the analysis. After sample clean up, salivary sample was centrifuged, after which the supernatant was analyzed.

The 100 μL volume of salivary sample supernatant was analyzed by the Ecologiena® supersensitive BPA ELISA KIT (Japan EnviroChemicals, Ltd., Tokyo, Japan) to quantify the BPA levels and for high accurate determination, replicate measurements was conducted on samples. To the antibody-coated microplates, 100 μL of standard BPA or sample with conjugate solution was added at room temperature for 1 h. The reaction solution was removed and plates were washed three times with 300 μL of washing solution. One hundred microliters of color solution was added to each well for 30 min. The reaction was stopped by adding 100 μL of stop solution. The optical density (OD) was measured using an ELISA plate reader with a 450-nm filter.

The BPA levels before and after the composite resin filling procedure were compared to examine whether or not salivary BPA increased after treatment. Additionally, the level of BPA in the saliva collected 7 days after the procedure was compared with the levels of salivary BPA before and 5 min after the procedure to investigate whether or not BPA leaching is persistent.

Difference in the levels of BPA in the saliva collected before the