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Effects of Bisphenol A Released From Composite Fillings on Reproductive Hormone Levels in Men

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ABSTRACT

Objectives: Composite resins are the most preferred filling material because of their excellent aesthetic qualities. However, a filling material should also be biocompatible as well as aesthetic. The aim of this study was to determine the serum and saliva bisphenol-A (BPA) levels and to examine the effects of serum BPA on reproductive hormone levels after healthy men were treated with composite fillings.

Methods: Eighteen healthy males each received 2 composite restorations. Saliva and blood samples of subjects were collected before resin application and 1 day and 1, 3, and 5 weeks after the resin was applied. BPA amounts in samples were detected using high-performance liquid chromatography (HPLC). Serum gonadotropins, testosterone, sex hormone binding globulin, free androgen index, and oestrogen levels were measured with radioimmunological assay kits. Statistical analysis of data was made using Friedman, Wilcoxon signed ranks and Mann-Whitney U tests ($\alpha = 0.05$).

Results: The amount of BPA released from composite resins over time was not significantly elevated in either saliva or serum ($P > 0.5$). In addition, serum BPA levels were significantly higher than saliva BPA levels for both composites ($P < .05$), but saliva and serum BPA levels were not statistically different when comparing the 2 composites ($P > .05$).

Conclusions: BPA from composite resins used in this study did not significantly alter serum hormone levels.

Introduction

Bisphenol A (BPA: 2,2-bis(-hydroxyphenyl) propane, CAS No: 80-05-7) is an industrial chemical that was synthesized in 1891 for the first time and was found in the 1930s to have oestrogenic effects. With an annual production of more than 2 million tons today, BPA is the main monomer used in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used in the manufacturing of dental resins, baby bottles, food storage containers, water bottles and bottle caps, glass lenses, CDs, DVDs, and electronic devices.[1,2] Exposure to BPA is thought to have side effects on human health, particularly during infant development, which is a considerable public health problem. However, there are few basic studies on its possible effects on human health.[1-3]

Composite resins are the most preferred filling material today because of their excellent aesthetic qualities. The composite resins used in dentistry consist of resin matrix (organic phase) and fillers. Resin is the chemically active component of the composite. The most widely used monomers in resin matrix are urethane dimethacrylate (UDMA), bisphenol A glycidyl methacrylate (BISGMA), and triethylene glycol dimethacrylate (TEGDMA).[4]

Pure BPA is not found in dental products. BPA, BISGMA, and bisphenol A dimethacrylate (BISDMA) are the major resin monomers. If synthetic reactions cannot be completed

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cytokiometrically during the production of dental sealants containing BISGMA, BPA may be present in the product in an impure form. Composite resins are exposed to mechanical, bacterial, or thermal biodegradation in the oral cavity.[5] BPA can also be found as a degradation product of BISDMA via salivary esterases that can hydrolyse the ester bond found in BISDMA monomers.[6] As a result, BPA is absorbed into the blood from the gastrointestinal tract and redistributed to other tissues and conjugated in the liver in high doses to create BPA glucuronide, an important metabolite excreted in urine.[7,8]

BPA is thought to be an endocrine-disrupting chemical with toxic effects on reproduction.[9] In animal studies conducted on rodents and in vitro studies, it has been shown that BPA has both oestrogenic and antiandrogenic effects.[10-12] Studies have also shown that BPA exposure may have some side effects such as decreased epididymal or testicular sperm count, decreased sperm motility and speed, decreased epididymal weight, impaired insulin signal and glucose homeostasis, decreased steroidogenesis in the testis, and reduced serum follicle-stimulating hormone (FSH) and testosterone levels in the male reproductive system.[13-18] However, these observations are largely based on animal studies or in vitro experiments.

Because of exposure to environmental BPA and oestrogenic activity in vitro and in vivo, the potential negative effects of BPA exposure on human reproductive health have been a source of concern and have become a topic for further study.[19] A number of epidemiological studies have been conducted on BPA levels measured in humans and its sources, and it has been concluded that dietary intake is the principal BPA source.[20] Once BPA enters the body, it acts as an oestrogen agonist and androgen antagonist and may disrupt normal cell function and affect human health.[11,21] However, only a few epidemiological studies have investigated the relationship between BPA exposure and health-related end points, and as a result, human studies on the possible health effects of BPA exposure are limited.[22]

The aim of this study was to compare the relationship between the levels of BPA released from composite fillings into the saliva and serum in healthy males, and serum FSH, luteinizing hormone (LH), testosterone (T), sex hormone binding globulin (SHBG), free androgen index (FAI), and oestrogen (E2).

The null hypotheses of the study are as follows:

1. There is no difference between the amounts of BPA released from composite resins into saliva or serum over time.
2. The amount of BPA released is similar between different composite types.
3. The amount of BPA released into saliva and serum is similar for each composite resin.
4. Serum hormone levels did not change over time compared to baseline levels.

Materials and methods

Subjects and study design

This study was carried out with the ethical approval of Atatürk University Faculty of Dentistry Ethics Committee (20.11.2014/032) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all individual participants included in the study. This study was carried out on a total of 18 male subjects aged 19 to 24 years (21.11 ± 1.32) who were admitted to Atatürk University, Faculty of Dentistry, Restorative Dentistry Clinic.

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: 80% power, 0.58 effect size, and α error at 0.05. A minimum sample size of 18 participants was assessed to be appropriate.

Inclusion criteria were as follows:

- No systemic diseases.
- Have not taken any medication in the last 3 months.
- No smoking or alcohol use.
- No bruxism or a habit of chewing gum frequently.
- No filling materials in the mouth and no periodontal disease.

In the study, 2 composite materials (Charisma [Heraeus Kulzer GmbH] and Grandio [VOCO GmbH]) were applied according to the manufacturer's instructions. Clearfil S3 Bond (Kuraray) was used as a bonding agent. The information about the applied materials is given in Table 1.

The restorative materials were applied according to the manufacturer's instructions. The composite filling materials were given an anatomical form by placing into cavities not exceeding 2 mm in depth as 1 piece (bulk method) and polymerized for 40 seconds by 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 about 1200 mW/cm2. During the polymerization process, the tip of the light source was kept as close as possible to the restoration. The intensity of the light device was measured using a radiometer (Hilux Ultra Plus Curing Units; Benlioglu Dental). Finishing and polishing operations were completed using discs (Sof-Lex; 3M ESPE Dental Products). The amount of

Table 1 – Details of investigated materials.

Materials	Content		Manufacturer	Lot No
	Matrikx	Filler		
Charisma	BISGMA, TEGDMA	58 vol%, 78 wt%, Barium aluminum, fluoride glass Silicium dioxide	Heraeus Kulzer GmbH, Hanau, Germany	010024
Grandio	BISGMA, TEGDMA, UDMA	71.4 vol%, 87 wt%, Glass-ceramic particles	VOCO GmbH Cuxhaven, Germany	1525390
Clearfil S3 Bond	BISGMA, HEMA	Colloidal silica	Kuraray, Okayama, Japan	8R0053

BISGMA, bisphenol A-glycidyldimethacrylate; HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.

composite resin applied to each individual was calculated using the method that Gul et al[23] used and the amount of applied composite resin was calculated in grams.

Individuals were asked not to eat on each test day and the fillings were applied between 9 am and 12 pm. Saliva and blood samples were taken prior to the application of the filling (baseline) and 1 day, 1 week, 3 weeks, and 5 weeks after the fillings were applied. Saliva samples were taken into sealed Eppendorf tubes, blood samples were taken into biochemical tubes and both samples were centrifuged at 3500 rpm for 10 minutes.[23,24] The obtained samples were stored at −80 °C until analysis. The samples were sent to Atatürk University Medical Faculty, Biochemistry Laboratory for BPA and hormone analysis.

High-performance liquid chromatography analysis

HPLC conditions: The high-performance liquid chromatography (HPLC) device (Agilent Technologies 1100 Series) was used with a UV detector (Agilent Technologies 1100 Series) and chromatographic separation was performed on a BRISA LC2 C18 (250 × 3 mm, 5 μm; Technochroma). The mobile phase solvents were acetonitrile (A) and water (B) (70 A: 30 B, v/v), flow rate of 1.0 mL/min, and detection wavelength was set at 270 nm. The sample injection volume was 20 μL and the column temperature was room temperature.

Preparations of stock, serum calibration, and quality control solutions: The high purity BPA standard (CAS NO: 80-05-7) obtained from Sigma Aldrich was used for HPLC analysis. The stock solution of BPA (0.1 mg/mL) were prepared in acetonitrile. This solution was diluted to give the intermediate stock solution (1000 ng/mL). The calibration and quality control (QC) solutions for both saliva and serum were prepared by spiking into blank human serum and saliva with different standard BPA solutions to give final concentrations for BPA of 0.2-20 ng/mL for calibration and 0.5, 5, and 15 ng/mL for QC.

Extraction procedure of BPA from serum: This method was modified from the extraction method developed by Kardas et al[25]: 1 mL of human serum was placed in the Eppendorf tube and later, 200 μL of CH_3COONH_4 buffer (0.01 M, pH: 4.5) and 5 mL mixed solvent of n-hexane and diethyl ether were added. The mixture was briefly vortexed and 5 mL n-hexane and diethyl ether was added twice. The solution was vortexed for 5 minutes, centrifuged at 5000*g* for 5 minutes, and the supernatant layer was transferred and evaporated to dryness under nitrogen stream. The resulting samples were dissolved in 100 μL acetonitrile and injected (20 μL) into the HPLC system.

Extraction procedure of BPA from saliva: This method was modified from the extraction method developed by Demirkaya and Kadioglu[26]: 1 mL of sample was mixed with 0.5 mL of acetonitrile, vortexed for 1 minute and mixed in the shaker for 15 minutes at room temperature. Then, the solutions were centrifuged at 1200*g* for 10 minutes. The supernatant layer was separated and injected (20 μL) into the HPLC system.

Hormone analysis

E2, FSH, LH, testosterone, SHBG, and FAI levels in the obtained serum samples were measured according to the manufacturer's recommendations using commercial immunoassay kits (Beckman Coulter Inc.). E2 (Lot No: 671144) levels were determined in pg/mL, FSH (Lot No: 671161) and LH (Lot No: 771005) levels were in mIU/mL, testosterone (Lot No: 631414) levels were in ng/mL, and SHBG (Lot No: 630345) levels were determined in nmol/L. FAI levels were calculated as a % (testosterone × 100/SHBG).

Reference values were taken as 20-47 pg/mL for E2, 1.27-19.26 mIU/mL for FSH, 1.24-8.62 mIU/mL for LH, 1.75-7.81 ng/mL for testosterone, and 14.5-48.4 nmol/L for SHBG.

Statistical analysis

The data obtained were analysed using SPSS 18 statistical package software (IBM). First, the Kolmogorov-Smirnov test was used to determine whether the data showed normal distribution. The Friedman test was used to compare the amount of BPA levels released from both composites into saliva and serum over time and the changes in serum hormone levels during these time periods. The Wilcoxon signed rank test was used to determine the difference between groups in case of a difference between groups. The Mann-Whitney U test was used to compare the composite resins in terms of BPA amounts released. In addition, Wilcoxon signed ranks test was used to compare the amount of BPA released into saliva and serum for each composite resin. The Spearman correlation test was used to determine the relationship between weights of composite resins and the amount of BPA released into saliva and serum, as well as any relationship between serum BPA levels and hormone levels. Statistical significance was established as $P < .05$.

Results

HPLC diode-array detection (DAD) analysis, which is a powerful quantification method, was used in this study to determine the BPA level in serum and saliva. BPA showed the maximum peak at 270 nm and the retention time was 5.7 minutes according to the chromatograms. The chromatogram obtained from blank serum containing endogenous BPA and serum spiked with 1 ng/mL BPA are given in Figures 1A and B, respectively. The same way, the chromatogram obtained from blank saliva containing endogenous BPA and saliva spiked with 1 ng/mL BPA are shown in Figures 2A and B, respectively. There exists endogenous BPA in the pooled blank serum and saliva. So, standard addition method for the calibration curve was used in serum and saliva to determine endogenous levels of BPA. The different BPA concentrations were added into both blank serum and blank saliva. Calibration curves were obtained by plotting between peak areas and added BPA concentrations. The equations for calibration curves for both serum and saliva were obtained as $y = 1.4571x + 0.6789$ (R^2 :0.9986, *n*:6) and $y = 1.4952x + 0.6390$ (R^2: 0.9992, *n*:6) in the 0.2-20 ng/mL concentration range for determination of BPA and the endogenous BPA for both serum and saliva were calculated to be 1.12 ng/mL and 0.4 ng/mL, respectively.

The method precision and accuracy were achieved by analyzing the intra-day (6 times per day) and inter-day (6 times

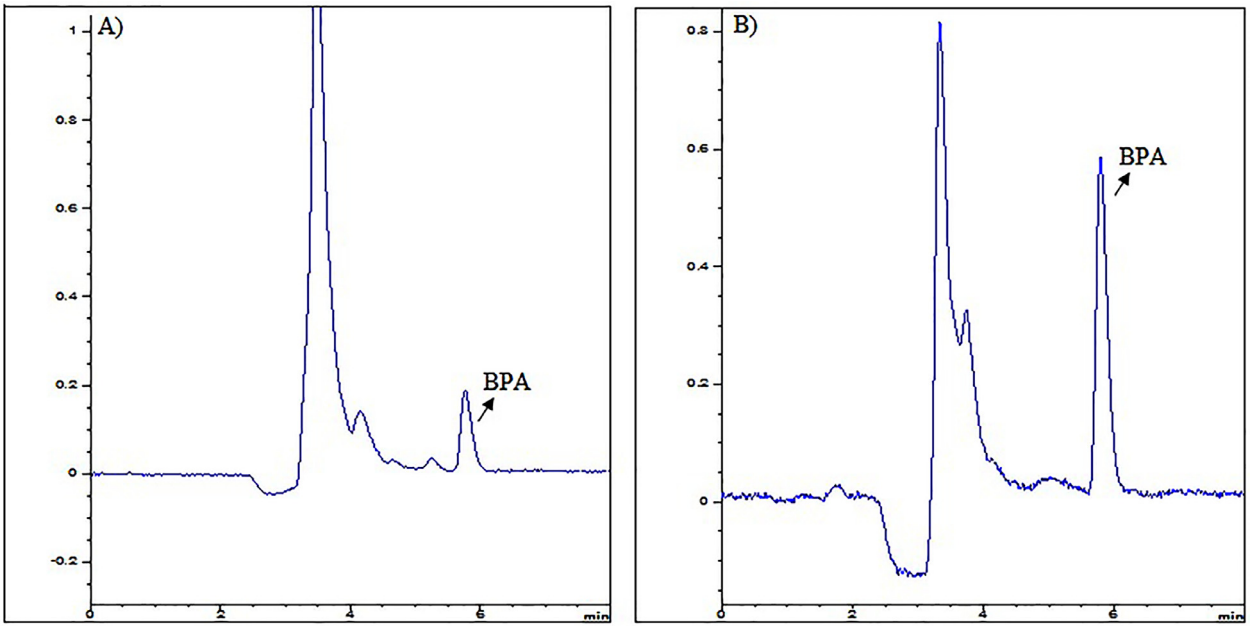

Fig. 1 – HPLC-DAD chromatograms (A) Blank serum (1.12 ng/mL endogenous BPA) (B) Blank serum spiked BPA (1 ng/mL). BPA, bisphenol A; HPLC-DAD, high-performance liquid chromatography diode-array detection.

once daily for 6 days) on QC samples (0.5, 5 and 15 ng/mL). The precision of the method for serum and saliva medium was given by the Relative Standard Deviation (% RSD) which calculated as 2.46% and 1.93%, respectively. The accuracy of the method was given by Percent Relative Error (RE%) and found as ±3.81% and ±2.45% for serum and saliva medium, respectively. The extraction recoveries of BPA in serum and saliva medium were determined to be 98.4 ± 4.21% and 99.5 ± 3.87% for all calibration concentration, respectively. According to the results, the method is accurate and precise for determination of BPA level in serum and saliva medium. The limit of quantification (LOQ) were calculated as $10 \times \sigma$ (standard deviation of y-intercepts)/S (slope of the calibration curve) and found as 0.15 ng/mL for each medium. The developed and validated HPLC method was applied to quantify BPA in both serum and saliva samples of volunteers who exposure two different composite materials along 0 (baseline), 1 day, 1, 3 and 5 weeks. The obtained chromatograms

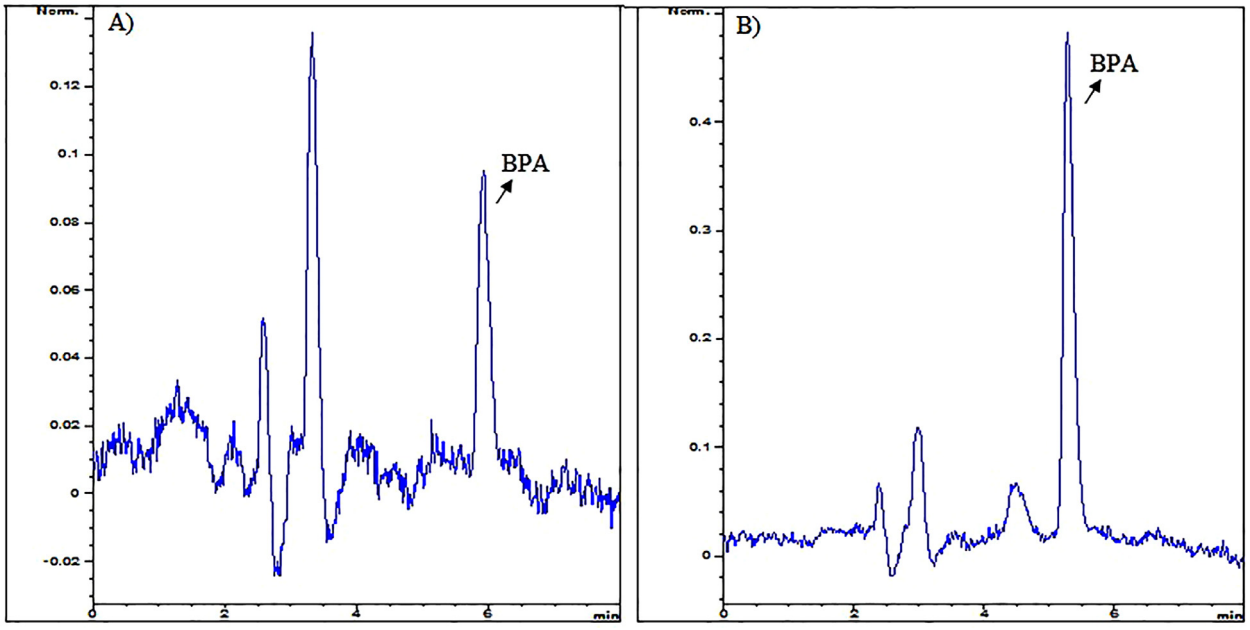

Fig. 2 – HPLC-DAD chromatograms (A) Blank saliva (0.4 ng/mL endogenous BPA) (B) Blank saliva spiked BPA (1 ng/mL). BPA, bisphenol A; HPLC-DAD, high-performance liquid chromatography diode-array detection.

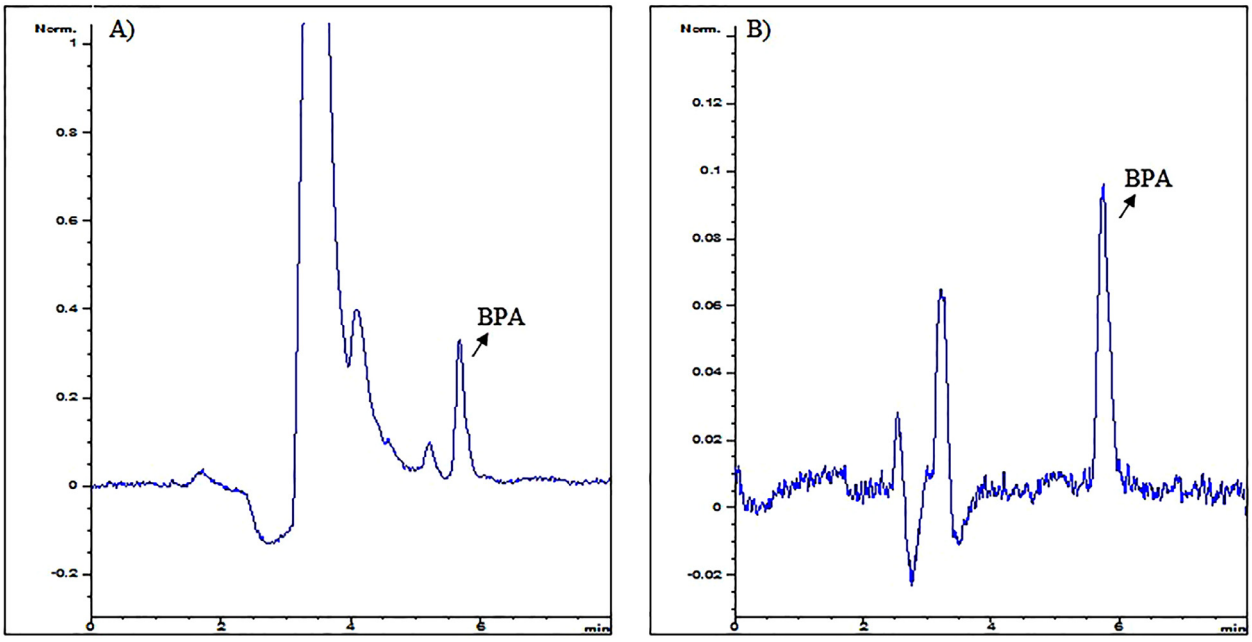

Fig. 3 – HPLC-DAD chromatograms of the obtained from the subject after treatment for (A) serum (1.68 ng/mL BPA) (B) saliva (0.47 ng/mL BPA). BPA, bisphenol A; HPLC-DAD, high-performance liquid chromatography diode-array detection.

from the subject after treatment for serum and saliva BPA were given the Figure 3.

Table 2 shows the BPA levels released from 2 different composite resins into saliva and serum over time, the serum hormone levels measured in these time periods, and the results of the statistical comparison. As a result of the Kolmogorov Smirnov test on the distribution of the obtained data, it was determined that the data were not normally distributed ($P < .05$).

Although the amount of BPA released from the composite resins was less than that observed in saliva, it was increased compared to baseline levels; however, this difference was not statistically significant ($P > .05$). In terms of the amount of BPA released into serum, it was found that the amounts of BPA released over time in both composite resins did not show a significant increase ($P > .05$). The Wilcoxon signed rank test performed to compare the levels of BPA in serum and saliva showed that the amount of BPA released in both composite resins was statistically significantly higher in serum compared to the amount released in the saliva ($P < .05$). Based on the results of the Mann-Whitney U test performed to compare composite resins in terms of amounts of BPA released into saliva and serum, no statistically significant difference was found between the 2 composite resins ($P < .05$).

When serum hormone levels were analysed over time after composite resins were applied, no significant difference was found in both composite resins in terms of E2 in all time periods ($P > .05$). When the measured amounts of FAI, FSH, and LH after application of both composite resins were compared, the levels of all 3 hormones significantly decreased compared to baseline for Grandio ($P < .05$), but this decrease was not statistically significant for Charisma ($P > .05$). Although there was an increase in testosterone, this was not significant compared to baseline ($P > .05$). SHBG increased in comparison with baseline, but this increase was not statistically significant ($P > .05$). Decreases and increases in the hormone levels did not go beyond the normal reference intervals in all time periods.

The mean filling weights were 0.05 ± 0.03 g and 0.10 ± 0.09 g for Grandio and Charisma, respectively. There was no statistically significant correlation between the filling weights and the BPA amounts released into serum and saliva for both composite resins ($P > .05$). In addition, there was no statistically significant correlation between BPA levels released into serum from both composite resins and hormones levels ($P > .05$).

Discussion

Composite resins are the most preferred filling material because of their excellent aesthetic qualities. However, a filling material should also be biocompatible as well as aesthetic. Biocompatibility is the ability of a material to be compatible with the biological functions of the tissue without showing toxic and harmful effects. Ideally, the dental materials used in the mouth should be harmless for oral tissues. Moreover, they should not cause systemic or local toxicity, mutagenicity, or carcinogenic effects.[4,27]

In this study, saliva and serum BPA levels were measured after 2 different composite resins were applied to healthy male subjects, and the effect of BPA on reproductive hormones was investigated. The HPLC method was used to determine the amount of BPA in saliva and serum samples. The HPLC technique is the most suitable method for eluting the nonpolar compounds forming the composite resin monomer and also has the advantage of separating the

Table 2 – BPA levels released from 2 different composite resins into saliva and serum over time, the serum hormone levels measured in these time periods, and the results of the statistical comparison.

Materials	Parameters	Baseline	1 day	1 week	3 weeks	5 weeks
Grandio	BPA saliva (ng/mL)	0.29 ± 0.19^{A}	0.46 ± 0.23^{A}	0.52 ± 0.54^{A}	0.49 ± 0.29^{A}	0.34 ± 0.24^{A}
	BPA serum (ng/mL)	1.42 ± 0.31^{A}	1.17 ± 0.34^{AB}	1.21 ± 0.31^{AB}	1.01 ± 0.16^{B}	1.49 ± 1.04^{AB}
	E2 (pg/mL)	36.00 ± 13.26^{A}	30.10 ± 18.81^{A}	40.70 ± 15.18^{A}	33.60 ± 16.24^{A}	33.70 ± 19.48^{A}
	FSH (mIU/mL)	4.38 ± 2.95^{A}	4.23 ± 3.56^{AB}	3.88 ± 2.78^{ABC}	3.70 ± 2.78^{BC}	3.55 ± 2.68^{C}
	LH (mIU/mL)	4.66 ± 2.44^{AB}	4.22 ± 2.36^{AB}	4.40 ± 1.91^{AB}	3.80 ± 1.29^{B}	2.86 ± 1.9^{C}
	Testosterone (ng/mL)	3.66 ± 0.82^{A}	3.94 ± 0.64^{A}	4.04 ± 0.80^{A}	4.67 ± 0.98^{B}	3.76 ± 1.42^{A}
	SHBG (nmol/L)	21.27 ± 15.36^{A}	22.61 ± 15.77^{A}	21.54 ± 14.41^{A}	21.89 ± 14.15^{A}	24.63 ± 12.27^{A}
	FAI	0.71 ± 0.27^{AB}	0.70 ± 0.22^{AC}	0.78 ± 0.28^{AB}	0.85 ± 0.31^{B}	0.58 ± 0.26^{C}
Charisma	BPA saliva (ng/mL)	0.20 ± 0.15^{A}	0.31 ± 0.27^{A}	0.33 ± 0.22^{A}	0.31 ± 0.23^{A}	0.28 ± 0.14^{A}
	BPA serum (ng/mL)	1.27 ± 0.24^{A}	0.98 ± 0.08^{B}	1.39 ± 0.59^{AB}	1.06 ± 0.14^{AB}	1.66 ± 0.78^{A}
	E2 (pg/mL)	33.75 ± 17.74^{A}	34.25 ± 12.77^{A}	31.38 ± 21.39^{A}	31.75 ± 11.30^{A}	37.00 ± 17.27^{A}
	FSH (mIU/mL)	5.24 ± 2.98^{A}	4.95 ± 2.49^{A}	5.01 ± 2.86^{A}	4.96 ± 2.74^{A}	4.91 ± 2.70^{A}
	LH (mIU/mL)	4.07 ± 1.80^{AB}	3.99 ± 1.35^{A}	3.54 ± 1.35^{A}	4.96 ± 0.89^{B}	3.82 ± 1.47^{AB}
	Testosterone (ng/mL)	3.66 ± 0.60^{AC}	3.51 ± 0.79^{AB}	3.15 ± 0.66^{B}	3.69 ± 0.91^{ABC}	3.99 ± 0.75^{C}
	SHBG (nmol/L)	16.71 ± 4.24^{A}	15.69 ± 4.61^{B}	17.10 ± 5.15^{A}	18.49 ± 7.01^{AC}	19.65 ± 6.92^{C}
	FAI	0.80 ± 0.16^{A}	0.86 ± 0.24^{A}	0.66 ± 0.20^{B}	0.78 ± 0.29^{AB}	0.76 ± 0.19^{A}

BPA, bisphenol A; E2, oestrogen; FAI, free androgen index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin.
Data shown are mean ± SD. Different uppercase letters indicate a significant difference for values within that row.

components according to their hydrophobic order.[28] Also, because the monomers can be dissolved in the mobile phase in the HPLC method, the separation process is carried out at a more controlled level. The molecules consisting of high-molecular-weight monomers such as BISGMA can be decomposed in the gas chromatography technique, and only decomposition products can be detected. Therefore, the HPLC method is more suitable for determining the type and amount of monomers released from composite resins. For these reasons, the amount of monomer released from the composite resins in present study was measured using the HPLC method.[29]

In recent years, following the application of fissure sealant and composites, the results of studies evaluating the levels of BPA, BISGMA and BISDMA in saliva have been compared.[2,30,31] BPA derivatives have been detected in some studies conducted in vitro and in vivo.[30,31] In this regard, Lewis et al[32] measured BPA release from 28 commercially available dental products (20 composites, 8 sealants) using HPLC and indicated that the amount of released BPA was too small to be measured. As a result, researchers concluded that dental resins could not be a source of BPA. Fleisch et al[2] stated that BPA can be detected in saliva for 3 hours after the resin is placed. In their study, Sasaki et al[33] applied 9 different composite resins (Z100, Progress, Palfique, Metafile Flo, UNIFIL S, Beautifil, Xeno CFII, Prodigy, Clearfil ST) to 21 individuals and found that the BPA amounts released into saliva varied between 15 and 100 ng/mL. They also found that gargling for 30 seconds after polymerization significantly reduced the rate of BPA release. In another study, Kingman et al[34] measured the amount of BPA released into saliva before and after composite resin treatment. Researchers have argued that BPA detected in saliva may be a good indicator of BPA released from resin because it is possibly the highest amount that can be measured because saliva is in direct contact with the resin. Researchers have stated that the amount of BPA measured before resin application is basal BPA and that the amount of BPA measured after resin application may be the amount of BPA directly associated with resin. In their study, Kingman et al[34] found the level of BPA measured before resin application was 0.43 ng/mL, 0.64 ng/mL after 1 hour, and 0.41 ng/mL after 9 to 30 hours. The BPA rate returned to baseline after 30 hours.

Because the aim of this study was to measure the amounts of BPA released after resin application, we aimed to measure BPA levels in saliva, which is the first point of contact with the resin, and BPA levels in serum were also measured to examine the effect on the hormonal system. We found higher serum BPA levels than saliva BPA levels may be attributed to the continuous ingestion of saliva and the cumulative effect of basal BPA levels. The fact that this condition continues during the follow-up period supports our claim.

In many studies where serum BPA levels were measured using different analytical techniques, human serum BPA levels were found to vary between 0.2 and 20 ng/mL.[35] However, the data recorded in biological monitoring studies show that the estimated nonmetabolized BPA in human blood or serum samples is in a steady state in the range of 0.5-3 ng/mL (2-13 nM).[35,36] When we look at studies on the cytotoxic doses of monomers released from composite resins, this dose was found to be 0.01 mmol/L (10000 nM) for BPA.[37] Studies have reported that BPA released from dental materials is minimal and not at a level that can pose a health risk.[38,39]

After the composite resin was applied, BPA released from composite resins into saliva and serum were measured at certain intervals up to 5 weeks, and the maximum value measured in saliva was 0.52 ng/mL, whereas the maximum value measured in serum was 1.66 ng/mL. Looking at the results obtained, it was found that even the highest BPA levels released into saliva and serum among all time intervals were below the levels indicated in the literature. In this respect, the data obtained in the present study are consistent with the mentioned literature.

Although BPA values in saliva and serum increased initially, this increase was not significant. Moreover, the

amounts of released BPA were similar for both composite resins ($P > .05$). Conversely, Joskow et al[30] concluded that BPA exposure after Delton LC sealant placement was significantly higher than exposure after placement of Helioseal F. Patients treated with Delton LC had significantly higher doses of BPA (110 μg) than did those treated with Helioseal F (5.5 μg) ($P < .0001$). As a result, the first and second hypotheses of the present study were accepted, but the third hypothesis was rejected because the amount of BPA released into the serum from composite resins was significantly higher than BPA released into saliva ($P < .05$).

In the literature, researchers also used urine to quantify BPA exposure in humans. Urinary BPA (uBPA) concentrations increased 24 hours after dental treatment. The 2 studies with the largest sample sizes[34,40] found statistically significant increases >40% in uBPA concentrations at 24 hours posttreatment (both P values $< .01$). The 1 study that examined uBPA concentrations beyond 1 month posttreatment found that concentrations returned to baseline by 14 days after treatment and remained at baseline 6 month after treatment. [40]

It is known that gonadal hormones affect human reproductive activities. When considered in terms of mechanisms of action, FSH induces spermatogenesis through receptors in Sertoli cells found in the seminiferous tubules of the testis.[41] Testosterone is secreted by the adult Leydig cells in men and is mainly regulated by the LH. The majority of serum testosterone is bound to SHBG. SHBG is a glycoprotein responsible for the transport of testosterone and oestradiol in the bloodstream.[42,43] SHBG concentration increases with oestrogen and decreases with androgen. For this reason, SHBG production is stimulated by oestradiol and is blocked by testosterone. It is clear that exposure.[44] To endocrine disruptors that affect gonadal hormone levels may cause changes in the reproductive performance of the individual. For this reason, the relationship between the BPA released from composite resins and reproductive hormones was also examined in the present study. When considered in terms of the role of hormones on the human reproductive system, studying the rate of release of a chemical that can affect the endocrine system, and its relationship with the hormones contributes to the importance of our study.

Studies in the literature are usually based on the examination of the effect of BPA on the human endocrine system resulting from environmental sources. Based on our literature survey, our study is the first to examine the effect of BPA, which is released from composite resins into saliva and serum, on reproductive hormones. When we look at the studies investigating the relationship between BPA and reproductive hormones in humans, Hanaoka et al[45] reported that uBPA levels of 42 workers exposed to BPA were significantly higher than the controls and that there was also an inverse relationship between uBPA levels and serum FSH levels. In their study, Meeker et al[46] found a positive correlation between BPA levels and serum FSH and a negative correlation between BPA and LH, FAI, and E2 in urine specimens taken from 167 male subjects. Mendiola et al[47] investigated the relationship between uBPA levels and serum hormones in fertile men and found a negative relationship between BPA and FAI and a positive relationship between BPA and SHBG. Takeuchi et al[48] found that there was a positive relationship between serum BPA levels and serum total and free testosterone levels in 11 male subjects. Zhou et al[49] found that serum levels of BPA were negatively correlated with serum free testosterone and FAI levels, whereas there was a positive correlation between BPA and serum SHBG levels. Cha et al[50] found a positive relationship between BPA and LH but a negative relationship between BPA and FSH in individuals with work-related BPA exposure. Lassen et al[51] conducted a study on 308 young male subjects and found a positive relationship between BPA and LH but a negative relationship between BPA and FSH. As can be seen in the literature, the results differ between studies. This disparity can be explained by the differences in the populations in which the studies were carried out, the number of individuals, and the measurement methods of the samples.

With respect to the endocrine system, we found that BPA does not activate the endocrine system, and the minimal changes that occur in hormone levels do not go beyond the reference values. In this respect, the fourth hypothesis has been partially accepted. In addition, there was no statistically significant correlation between the released BPA levels and hormone levels ($P > .05$). Given the results of our study, the amounts of BPA released from the resins used are well below the toxic doses. This may explain why the endocrine system is not activated.

The limitation of this study is the small number of individuals in the study sample because of the difficulty of finding individuals who fit the inclusion criteria. In addition, diets of the participants were not changed. However, the fact that the samples were repeatedly taken at specific time intervals instead of a single sample makes our study valuable in terms of monitoring BPA levels. However, there is a need for studies with larger samples to confirm the results.

Conclusions

BPA was found in both serum and saliva released from composite resins. However, it was determined that the released BPA levels were below the toxic doses and did not alter hormonal balance. Additionally, there is a need for studies involving different materials and a larger number of individuals.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Neslihan Celik, Fatma Betul Ozgeris, and Fatma Demirkaya-Miloglu. The first draft of the manuscript was written by Pinar Gul and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

None disclosed.

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