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Research / Araştırma Makalesi

Chlorophyll, Carotene, and Anthocyanin Effect on Color Change of Resin-Based Dental Materials: An *in-Vitro* Study

Klorofil, Karoten ve Antosiyaninin Rezin Bazlı Dental Materyallerdeki Renk Değişimine Etkisi: *in Vitro* Çalışma

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ABSTRACT

Background: The purpose of the present study was to evaluate the effect of chlorophyll, carotene, and anthocyanin-containing juices on color stability of resin-based dental materials.

Methods: 192 disc-shaped specimens were prepared from six resin-based materials (universal, anterior, and flowable resin composites; self-curing polymethyl methacrylate (pmma), bis-acrylic composite, and computer-aided design/computer-aided manufacturing(CAD/CAM)- fabricated pmma). After immersion in distilled water at 37ºC for 24 hours, randomly divided groups were immersed in distilled water, fresh spinach, carrot, and black mulberry juices (n=8). Specimens were continuously immersed in solutions which were renewed every day for 4 weeks. Color measurements were taken with a spectrophotometer before the immersions(T0), after one week(T1), and after four weeks(T2). The color differences (ΔE_{00}) were used for statistical analysis. ANOVA was used to determine the effects of material, solution, and time on color change.

Results: The highest color change was recorded for the CAD/CAM-fabricated pmma (ΔE=2.67) ($p<0.05$). Distilled water was the least coloring solution (ΔE=0.60), and carrot juice was the highest (ΔE=2.37). No significant difference was seen between black mulberry and carrot juice (p=0.071). The mean ΔE value was 1.84 in T0-T1 and 1.43 in T1-T2 ($p<0.001$).

Conclusions: Carrot was the highest colorant with black mulberry, giving clinically unacceptable ΔE_{00} values. Spinach caused only visually perceptible coloration. All materials got visually perceptible color change after the juice immersions. Only CAD/CAM-fabricated pmma showed clinically unacceptable ΔE_{00} values.

Keywords: Composit resins; coloring agents; dental restoration temporary; dental restoration permanent, dental materials

ÖZ

Amaç: Bu çalışmanın amacı; klorofil, karoten ve antosiyanin içeren bitki sularının, rezin bazlı dental materyallerin renk stabilitesi üzerindeki etkisinin ölçülmesidir.

Gereç ve Yöntemler: Altı farklı rezin bazlı dental materyalden (universal, anterior ve akışkan rezin kompozit; kendi kendine sertleşen polimetil metakrilat (pmma), bis-akrilik kompozit ve bilgisayar destekli tasarım/bilgisayar destekli üretim (CAD/CAM) ile üretilen pmma) 192 disk şeklinde örnek hazırlandı. Örnekler 24 saat boyunca 37ºC distile suda bekletildikten sonra rastgele gruplara ayrıldı ve distile su, taze sıkılmış ıspanak, havuç ve karadut sularına (n=8) daldırıldı. Örnekler hergün yenilenen solusyonlarda 4 hafta boyunca bekletildi. Daldırmalardan önce (T0), bir hafta sonra (T1) ve dört hafta sonra (T2) spektrofotometre ile renk ölçümleri yapıldı. İstatistiksel analiz için renk farklılık değerleri (ΔE_{00}) kullanıldı. Materyalin, solüsyonun ve zamanın renk değişimi üzerindeki etkilerini belirlemek için ANOVA kullanıldı.

Bulgular: En fazla renk değişimi CAD/CAM ile üretilen pmma'da kaydedildi (ΔE=2.67) ($p<0,05$). En az renk değişimi distile suda (ΔE=0.60), en yüksek renk değişimi havuç suyunda kaydedildi (ΔE=2,37). Karadut ve havuç suyu arasında anlamlı fark görülmedi (p=0.071). Ortalama ΔE değeri T0-T1'de 1.84, T1-T2'de 1.43 idi ($p<0,001$).

Sonuç: Havuç, karadut ile birlikte en yüksek renklendirici olup, klinik olarak kabul edilemez ΔE_{00} değerleri verdi. Ispanak yalnızca görsel olarak algılanabilen bir renklenmeye neden oldu. Tüm materyaller bitki sularında bekletildikten sonra görsel olarak algılanabilir bir renk değişimine uğradı. Yalnızca CAD/CAM ile üretilmiş pmma, klinik olarak kabul edilemez ΔE_{00} değerleri gösterdi.

Anahtar Kelimeler: Kompozit rezinler; boyayıcı ajanlar; geçici restorasyonlar; kalıcı restorasyonlar; dental materyaller

Introduction

The importance of healthy nutrition has become more evident as it is easier to reach information about which food is beneficial for what. It was reported that fruits and vegetables are primary sources of antioxidants.[1,2] Accordingly, the consumption of antioxidant drinks prepared by squeezing vegetables or fruits has also become popular.[3,4] The color of the juice can be very intense related to the coloring compounds like chlorophyll, carotene, or anthocyanin, which are pigments responsible for the green, orange, and violet colors of the plants respectively.[5]

Green vegetables that contain chlorophyll pigment are reported to be antioxidant and antimutagenic, preventing many diseases like cancer.[6]

Orange-colored vegetables containing carotenoids have antioxidant properties mainly due to pro-vitamin A activity preventing cardiovascular disease and cataracts.[5,7]

Anthocyanin pigment that gives plants red-violet color is highly antioxidant with anti-cancer, anti-diabetic, and anti-inflammatory activity.[8] Black mulberry, which is rich in anthocyanin pigment, has also been evaluated and recommended by several studies for treating oral mucositis due to the anti-inflammatory and analgesic effects of the plant.[9,10] The potential coloring effect for dental structures may also become more important if studies supporting the use of black mulberry increase.

The color change of tooth-colored dental resins caused by daily consumption of drinks has been evaluated in many studies.[11-15] It was reported that color change was observed, in time, for both temporary acrylic [13-15] and composite-based resin materials after various solution immersions.[11,12,16]

Spectrophotometers are widely used as color-measuring devices for evaluating the color change of dental structures.[11] Easyshade (Vita Zahnfabric) spectrophotometer was developed for dental use and is commonly used in studies 11 due to its highly accurate and reliable reports.[17] A spectrophotometer gives the color coordinate values needed for the color difference evaluations. Color differences have been measured by mainly two formulas of CIE L*a*b* and CIEDE2000.[11,12] CIE L*a*b* formula was defined previously by the Commission Internationale de l'Eclairage with L*, a*, and b* color coordinates.[18]

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Later, the CIEDE2000 formula came into use and was widely accepted due to the system's improved color equations.[11,19] Evaluated color difference (ΔE) values become important if the values are beyond accepted threshold values for acceptability and perceptibility. The ΔE threshold for acceptability is correlated with the color change regarded as clinically unacceptable. The threshold for perceptibility correlates with the color change the human eye can detect.[20,21] It was reported in a study that, for CIEDE2000 evaluation, the acceptability ΔE_{00} threshold value was 0.8, and the perceptibility threshold was 1.8.[21]

Studies found that red wine, black coffee, and black tea, which have intense red and dark brown colors, resulted in strong discoloration compared to most of the other tested beverages.12,16,[22] Although there have been many studies on frequently consumed beverages [11], the studies on the color stability of dental resins caused by antioxidant juices obtained from colored fruits and vegetables are limited.23 With the increased consumption of fresh antioxidant juices, intense green, orange, or red-violet colored drinks may have the potential as a strong colorant for dental restorative materials as well.

In the present study, spinach, orange-colored carrot, and black mulberry juice, known to contain intense chlorophyll, carotene, and anthocyanin pigments respectively were included in the study as colorant drinks.

The null hypothesis of the present study was that intense pigment containing fresh juices do not affect the color stability of various tooth-colored acrylic and resin composite materials.

Materials and Methods

Six groups of eight specimens each were prepared from three resin composites and three temporary acrylic resins. Materials are listed in **Table 1**.

Table 1. Materials used in the study.

	Resin Type	Composition	Manufacturer	Batch Number	Abbreviation
Filtek Z250	Micro-hybrid composite	BISGMA, UDMA, BISEMA, TEGDMA, zirconium/silica nonagglomerated particles	3M, ESPE	NC28785	FZ
Neospectra ST Flow	Flowable hybrid composite	Urethane modified BISGMA-adduct, BISEMA and diluents, camphorquinone, stabilizers, SphereTEC® fillers	Dentsply, Sirona	2010000269	NS
G-ænial Anterior	Micro-hybrid composite	UDMA, dimetharylate comonomers, prepolymerized fillers, SiO2, fumed silica	GC Corporation	20020051	GA
Temdent Classic	Self-curing acrylic	Polymethyl methacrylate	Schütz Dental	2018005004	TD
Protemp 4	Bis-Acrylic Composite	BISGMA, UDMA, TEGDMA, BISEMA, 50 nm silanized amorphous silica	3M, ESPE	4840421	PT
PMMA disc	CAD/CAM acrylic	Polymethyl methacrylate	Dentsply, Sirona	75477	PMD

BISGMA, Bisphenol A-glycidyl methacrylate; UDMA, Urethane dimethacrylate; BISEMA, Ethoxylated bisphenol-A dimethacrylate; TEGDMA, Triethylene glycol dimethacrylate

The immersion solutions were designed as distilled water, fresh orange-colored carrot, spinach, and black mulberry juice. All specimens, except for the computer-aided design/computer-aided manufacturing (CAD/CAM)-fabricated polymethyl methacrylate (pmma) group, were prepared in the same way using a silicone mold. A silicone mold was designed with eight disc-shape housings with the diameters of 7 mm and the heights of 2 mm (**Fig. 1**).

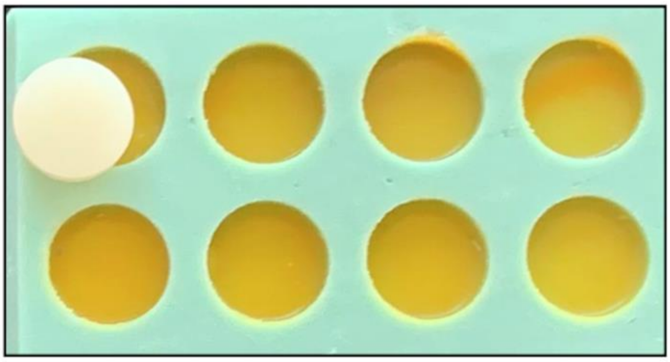

Figure 1. The silicone mold with disc-shape housings and an example of a specimen.

A bis-acrylic resin composite material (Protemp 4 Temporization Material A2, 3M ESPE)(PT) was injected into the housings via the cartridge mixing tip. A glass swab was put on the mold, and hand pressure was applied carefully to preserve the specimen dimensions and let the excessive material come out of the housings, leaving a flat surface. After waiting for the polymerization, the glass swab was removed, and the specimens were removed from the mold. A self-curing pmma-based acrylic resin (Temdent Classic, Schütz Weil-Dental)(TD) was prepared by mixing liquid and powder according to the manufacturer's recommendations. The mixture was poured into the housings, and after waiting under glass swab pressure, the specimens were removed from the mold when the polymerization was ended. A flowable hybrid resin composite (Neospectra ST Flow A2, Dentsply Sirona)(NS), a universal micro-hybrid resin composite (Filtek Z250 A2, 3M, ESPE)(FZ), and anterior micro-hybrid resin composite (G-ænial Anterior A2, GC)(GA) materials were adapted respectively filling half of the housings and light polymerized for 20 seconds using a LED curing device (Elipar DeepCure-S LED, 3M ESPE). After filling the remaining, the glass swab was covered under hand pressure and light cured for 20 seconds. The specimens were removed from the mold, and extra 20 seconds of light was given from the bottom surface of the specimens. For all mentioned specimens, excessive parts were removed to provide a smooth disc shape. The final acrylic resin group (PMD) was prepared from a pmma disc (Dentsply Sirona) with the CAD/CAM technology. With the exact dimensions, a disc-shape was designed in standard tessellation language (STL) format with an autodesk design program (Meshmixer). The STL file was transferred to the five-axis milling unit (inLab MC X5, Dentsply Sirona) and the specimens were produced from a pmma disc. 32 disc-shaped specimens of each material were prepared, and all surfaces were polished with a silicone polisher (OptraPol NG, Ivoclar Vivadent). All specimens were immersed in distilled water at 37 oC for 24 hours and divided randomly into four subgroups for the immersion solutions (n=8). The initial color measurements were taken with a spectrophotometer (Vita Easyshade V, Vita Zahnfabric) against a white background, and L*, a*, and b* values were recorded (**Fig. 2**).

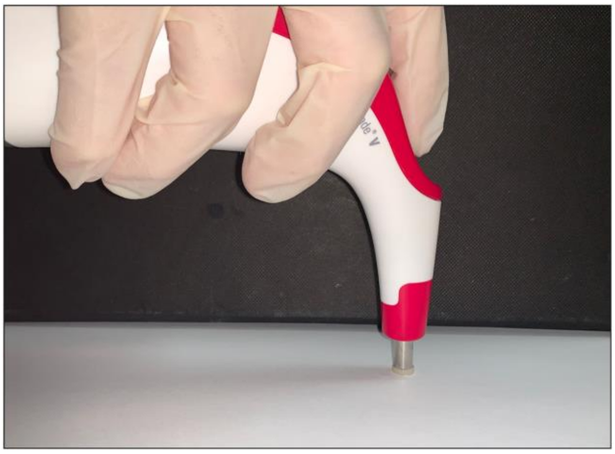

Figure 2. Representative image for color measuring of a specimen via dental spectrophotometer

Orange-colored carrot, spinach, and black mulberry juices were prepared with a juicer machine (The Nutri Juicer Cold, Sage). Eight specimens for each material group were immersed in distilled water (control), carrot, spinach, and black mulberry juices. Each specimen was stored in numbered 1.5ml micro-centrifuge tubes with clic-fit caps filled with the immersion solutions (**Fig.3**).

Figure 3. Specimens immersed in tested media (spinach juice, black mulberry juice, distilled water, carrot juice)

The solutions were changed every day with the fresh ones to avoid bacterial contamination and the specimens were rinsed under distilled water and dried with tissue paper before being immersed in fresh solutions. The second and third color measurements were taken after one and four weeks of continuous immersion. Before measurements, specimens were rinsed under distilled water for 30 seconds and dried with tissue paper.[12,15] All measurements were repeated three times. The mean L*, a*, and b* values were recorded. The calculations of color differences (ΔE_{00}) were done with the CIEDE2000 formula.[24]

The normality and homogeneity of the distribution of ΔE values were evaluated by the Shapiro-Wilk and Levene tests. Descriptive statistics were presented as mean and standard deviation. All statistical analysis were performed using a statistical software (PASW Statistics 22.0, SPSS, IBM). Repeated measures ANOVA was used to determine the effects of material, solution, and time on color change. The statistical significance level was set at 0.05.

In the present study, ΔE_{00} thresholds of perceptibility and acceptability were taken as 0.8 and 1.8, respectively.[21]

Results

The coloration varied depending on material, solution, and time ($p<0.05$) according to repeated measures ANOVA results. Color changes of all subgroups are shown in **Table 2**. While the highest color change was observed in PMD (ΔE=2.67), the other groups were ranked according to their mean ΔE values as follows: FZ (ΔE=1.78), GA (ΔE=1.54), TD (ΔE=1.38), PT (ΔE=1.34), and NS (ΔE=1.12). In pairwise comparisons between materials, there were statistical significant differences between TD and FZ (p=0.014), PT and FZ (p=0.004), NS and FZ ($p<0.001$), GA and NS (p=0.008). PMD had statistically significantly higher color change than all other groups ($p<0.05$). There was no significant difference between the other paired groups. When the effect of the immersion solution on the coloration of the material was examined, the least coloration was observed in distilled water (ΔE=0.60) and was significantly different from that in other solutions ($p<0.05$). The highest coloration was recorded in specimens immersed in carrot juice (ΔE=2.37). In pairwise comparisons between immersion solutions, there was no statistically significant difference between black mulberry and carrot juice (p=0.071). The p value was <0.001 among all other groups. It was observed that the time intervals affected the color change of the materials. While the mean ΔE value was 1.84 in T0-T1, this value was 1.43 in T1-T2 ($p<0.001$). The color changes of each material over time are presented in **Figure 4**.

Table 2. ΔE values (mean±standard deviation) of each group in T0-T1 and T1-T2 time intervals.(TD, Temdent Classic; PT, Protemp 4; PMD, PMMA disc; NS, Neospectra ST Flow; GA, G-ænial Anterior; FZ, Filtek Z250)

		T0-T1	T1-T2
TD	Distilled water	0.88±0.63	1.00±0.57
	Black mulberry juice	1.00±0.52	0.38±0.28
	Carrot juice	3.80±1.06	1.19±0.37
	Spinach juice	1.88±0.44	0.91±0.62
	Mean	1.89±1.36	0.87±0.55
GA	Distilled water	0.29±0.10	0.32±0.13
	Black mulberry juice	1.12±0.28	3.95±0.16
	Carrot juice	2.41±1.01	0.88±0.44
	Spinach juice	2.93±0.60	0.43±0.36
	Mean	1.69±1.21	1.40±1.54
PMD	Distilled water	0.16±0.07	0.20±0.05
	Black mulberry juice	0.35±0.11	11.75±0.14
	Carrot juice	4.58±1.39	1.71±0.79
	Spinach juice	1.72±0.40	0.84±0.90
	Mean	1.70±1.92	3.63±4.83
PT	Distilled water	1.49±0.14	1.38±0.11
	Black mulberry juice	0.67±0.23	0.23±0.11
	Carrot juice	3.41±1.98	1.56±0.81
	Spinach juice	1.52±0.40	0.41±0.33
	Mean	1.77±1.41	0.90±0.72
NS	Distilled water	0.36±0.20	0.30±0.09
	Black mulberry juice	1.21±0.37	0.28±0.13
	Carrot juice	1.62±1.13	1.43±0.30
	Spinach juice	3.05±0.90	0.72±0.49
	Mean	1.56±1.22	0.68±0.55
FZ	Distilled water	0.49±0.22	0.36±0.06
	Black mulberry juice	2.85±0.40	1.71±0.43
	Carrot juice	3.82±0.46	2.05±0.60
	Spinach juice	2.58±0.57	0.39±0.26
	Mean	2.44±1.30	1.13±0.86

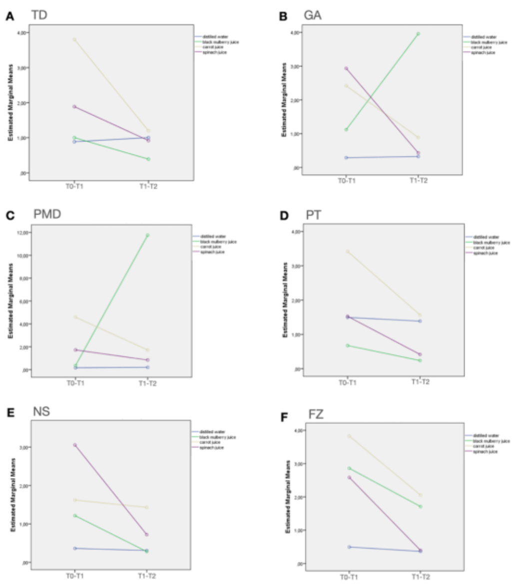

Figure 4. The change graph of mean ΔE values over time, A) Temdent, B) G-ænial, C) PMMA, D) Protemp, E) Neospectra, F) Filtek.

Discussion

The null hypothesis of the present study was rejected as the tested materials got color change after immersion in fresh juices containing intense pigments.

There has been a single study in the literature that evaluated the color change effect of green, orange, and red-colored fresh juices.[23] Differently, the immersion liquids of the study were mixtures of various juices and herbs. Mixing lots of ingredients may be diluted the pigments of the major coloring juice or change the ph that may lead to differences in color change. In the present study, the immersion solutions were freshly squeezed single substances. For obtaining the maximum coloring effect of chlorophyll, anthocyanin, and carotene, no other ingredients were added to the immersion solutions. The tested materials of the study of Yikilgan et al.[23] were three types of resin-based composites used for the anterior region. Consistent with the present study, the one common material (G-aenial Anterior) showed more color change than the two others (Amaris, Clearfil Majesty). In the present study, two other tested composites were a universal composite (Filtek Z 250) and a flowable resin composite (NeoSpectra ST Flow). Flowable composites were reported to have lower color stability due to lower filler content compared to universal composites.[25] However, in the present study, NS as a flowable resin composite showed lower color change (ΔE=1.12) than other packable resin composites. The material was reported by the manufacturer to have a patented SphereTec filler technology which was developed for easy application and enhanced esthetics.[26] However, to our knowledge, no other studies support the material's color stability outcomes.

In a study[22], four different resin composite materials' color changes were evaluated after immersion in artificial saliva, lemon juice, coffee, coca cola, sour cherry juice, fresh carrot juice, and red wine. According to the results, red wine caused the highest color change (ΔE=2.29). Red wine was reported to be a strong colorant in many other studies [12,27,28] however, red wine discoloration is not only due to the anthocyanin pigment but also due to the alcohol. [22,29] As a high source of anthocyanin pigment, black mulberry juice was the most coloring agent following carrot juice in the present study. Carrot juice was examined as an immersion solution in several studies.[22,30,31] Motayagheni et al.[30] evaluated the color changes of acrylic teeth with three different brands after immersion in carrot juice, orange juice, tea, and distilled water. Consistent with the present study, the most color change was seen in carrot juice immersed group after 7 (ΔE=3.69) and 30 (ΔE=4.65) days. Also, Antonov et al.[31] reported carrot juice as a high colorant for resin-based composites. In the present study, color change after immersion in carrot juice was the highest (ΔE=2.37), with no significant difference with black mulberry juice (ΔE=2.13). It was found that carrot juice as a carotene source and black mulberry juice as an anthocyanin source caused color change regarded to be clinically unacceptable (ΔE >1.8). Spinach juice as a chlorophyll source caused color change (ΔE=1.45) that is visually perceptible but clinically acceptable. The color change after immersion in distilled water was not perceptible for any groups.

In the present study, not only resin composites but also temporary acrylic resins were examined. There have been studies evaluating the color change of temporary resins after immersion in different solutions [13,14,32-35] since color change becomes essential, especially for the anterior region if the temporary restoration is planned to be used for the long-term.[34] Elagra et al.[32] evaluated the color stability of four temporary crown materials, including pmma, CAD/CAM-fabricated pmma, bis-acryl resin composite, and dual-curing resin composite. After immersion of tea solution for seven days, bis-acryl resin composite showed lower color stability than pmma, and CAD/CAM-fabricated pmma showed higher color stability than all other materials. Similarly, it was reported in another study that CAD/CAM-fabricated temporary crowns showed better color stability than temporary crowns that were prepared manually.[35] Although CAD/CAM-fabricated pmma products were reported to have enhanced color stability [32,35], in the present study, the most color change was seen in PMD group (ΔE=2.67). According to the threshold values, PMD group showed color change above the threshold value of "1.8", which is unacceptable for clinical conditions. It is to emphasize that the immersion solutions and used CAD/CAM-fabricated temporary acrylic material differed from previously mentioned studies. All other tested materials' color change levels were perceptible but clinically acceptable.

Although no correlation was reported between the surface roughness and color stability of resin-based composites immersed in green, orange, and red-colored detox juices with pHs 2.96, 3.82, and 2.72 respectively[23] , it is a limitation that the surface roughness of the specimens after immersion were not evaluated in the present study. Another limitation is that the methodology does not entirely simulate the intraoral conditions that may affect the color stability of the materials. Further studies are needed to evaluate intense pigmented fresh juices' in vivo coloring effect and the correlation of surface roughness changes due to pH differences.

Conclusions

The following conclusions could be drawn within the limitations of this in vitro study.

Spinach, orange-colored carrot, and black mulberry juices as the sources of chlorophyll, carotene, and anthocyanin pigments respectively, affected the color stability of resin-based dental materials. The coloring effect of carrot was the highest and similar to black mulberry with clinically unacceptable ΔE_{00} values. Spinach juice caused color change visually perceptible but in the range of clinical acceptability.

All resin materials got color change after juice immersions that would be visually perceptible. However, only CAD/CAM-fabricated pmma (PMD) showed clinically unacceptable ΔE_{00} values.

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Değerlendirme / Peer-Review

İki Dış Hakem / Çift Taraflı Körleme

Etik Beyan / Ethical statement

Bu makale, herhangi bir sempozyum ya da kongrede sunulan bir tebliğin içeriği geliştirilerek ve kısmen değiştirilerek üretilmemiştir.

Bu çalışma, yüksek lisans ya da doktora tezi esas alınarak hazırlanmamıştır.

Bu çalışmanın hazırlanma sürecinde bilimsel ve etik ilkelere uyulduğu ve yararlanılan tüm çalışmaların kaynakçada belirtildiği beyan olunur.

This article is not the version of a presentation.

This study has not been prepared on the basis of a master's/ doctoral thesis.

It is declared that during the preparation process of this study, scientific and ethical principles were followed and all the studies benefited are stated in the bibliography.

Benzerlik Taraması / Similarity scan

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Etik Bildirim / Ethical statement

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Finansman / Grant Support

Yazarlar bu çalışma için finansal destek almadığını beyan etmiştir. | The authors declared that this study has received no financial support.

Çıkar Çatışması / Conflict of Interest

Yazarlar çıkar çatışması bildirmemiştir. | The authors have no conflict of interest to declare.

Yazar Katkıları / Author Contributions

Çalışmanın Tasarlanması | Design of Study: BE (%60), BD (%40)
Veri Toplanması | Data Acquisition: BE (%50), BD (%50)
Veri Analizi | Data Analysis: BD(%100)
Makalenin Yazımı | Writing up: BE (%60), BD (%40)
Makale Gönderimi ve Revizyonu | Submission and Revision: BE (%60), BD (%40)

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