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The Effect of Photodynamic Therapy Applied with Different Photosensitizers on Dentin Hardness in Comparison with Conventional Irrigation

***Cihan Küden*[1-a*]**

[1] *Department of Endodontics, Faculty of Dentistry, Cukurova University, Adana, 01330, Türkiye*
**Corresponding author*

Research Article

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History

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ABSTRACT

Objectives: *The aim of this study was to examine the effect of photodynamic therapy (PDT) using toluidine blue O (TBO), curcumin (CUR) and methylene blue (MB) photosensitizers on root canal dentin microhardness by comparing it with sodium hypochlorite (NaOCl) + ethylenediamine tetraacetic acid (EDTA).*
Materials and Methods: *The root canals of 100 human premolar teeth were shaped by the R25 file (Reciproc; VDW, Munich, Germany). The working length of the teeth was determined by using a #10 K-file, keeping it 1mm shorter than the tooth apex. The R25 file was used to prepare the root canals. After every three pecking motions, irrigation was performed and a total of 10 mm of distilled water was used. The specimens were randomly distributed according to the disinfection method; NaOCl+EDTA, PDT with TBO, PDT with CUR, PDT with MB and distilled water (DS) (n=20). Grooves were prepared on the buccal and lingual surfaces of the prepared teeth, parallel to the long axis of the tooth, without touching the root canals. The roots were divided into two parts by means of a cement spatula placed in these grooves. Root canal dentin microhardness was evaluated by the Vickers test method. Three measurements were made by applying 300 g of force for 15 seconds and the average was calculated. It was recorded as the Vickers hardness value. The data were analyzed by using the one-way ANOVA and Dunnett's post hoc tests (α=0.05).*
Results*: All photosensitizer groups showed significantly higher microhardness value than the groups of DS and NaOCl + EDTA (p<0.05). There were no differences the groups of photosensitizers (p>0.05).*
Conclusions: *The use of 5.25% NaOCl solution activated by KTP laser, which shows the highest antibiofilm efficiency among the study groups, in clinical applications is very effective in terms of biofilm elimination in root canal treatments and is especially promising in the success of long-follow-up treatments. However, the KTP laser activation procedures of super-oxidized water solution and 8 ppm ozonated water may be insufficient as a safe disinfection method.*

Keywords: *PDT with CUR obtained the highest radicular dentin microhardness.*

Farklı Işığa Duyarlılaştırıcılar ile Uygulanan Foto Dinamik Terapinin Konvansiyonel İrrigasyon ile Karşılaştırılarak Dentin Sertliğine Etkisi

Bilgi
 # Bu çalışma 22-24 Kasım 2022 tarihleri arasında düzenlenen 'Sivas Cumhuriyet Üniversitesi 2. Uluslararası Diş Hekimliği Kongresi'nde sözlü bildiri olarak sunulmuştur.

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ÖZ

Amaç: *Bu çalışmanın amacı, toluidin mavisi O (TBO), kurkumin (CUR) ve metilen mavisi (MB) ışığa duyarlılaştırıcılar kullanılarak yapılan fotodinamik tedavinin (PDT) kök kanal dentin mikrosertliği üzerindeki etkisini sodyum hipoklorit (NaOCl) + etilendiamin tetraasetik asit (EDTA) ile karşılaştırarak incelemektir.*
Gereç ve Yöntemler: *100 adet insan premolar dişinin kök kanalları R25 eğesi (Reciproc; VDW, Münih, Almanya) ile şekillendirildi. Dişlerin çalışma uzunluğu, diş apeksinden 1 mm daha kısa tutularak #10 K-file kullanılarak belirlendi. Kök kanallarını hazırlamak için R25 eğesi kullanıldı. Her üç gagalama hareketinden sonra irrigasyon yapıldı ve toplam 10 mm distile su kullanıldı. Numuneler dezenfeksiyon yöntemine göre rastgele dağıtıldı; NaOCl+EDTA, TBO ile PDT, CUR ile PDT, MB ve distile su ile PDT (DS) (n=20). Hazırlanan dişlerin bukkal ve lingual yüzeylerine, dişin uzun eksenine paralel olacak şekilde, kök kanallarına dokunmadan oluklar hazırlandı. Bu oluklara yerleştirilen siman spatülü ile kökler iki parçaya bölündü. Kök kanal dentin mikrosertliği Vickers test yöntemi ile değerlendirildi. 15 saniye boyunca 300 g kuvvet uygulanarak üç ölçüm yapıldı ve ortalaması hesaplandı. Vickers sertlik değeri olarak kaydedildi. Veriler, tek yönlü ANOVA ve Dunnett'in post hoc testleri (α=0,05) kullanılarak analiz edildi.*
Bulgular: *Tüm ışığa duyarlılaştırıcı grupları, DS ve NaOCl+EDTA gruplarından önemli ölçüde daha yüksek mikrosertlik değeri gösterdi (p<0,05). Işığa duyarlılaştırıcı grupları arasında fark bulunmadı (p>0,05).*
Sonuçlar: *CUR ile PDT, en yüksek radiküler dentin mikrosertliği gösterdi.*

Anahtar Kelimeler: *Kürkümin, Mikrosertlik, Fotodinamik Tedavi, Işığa Duyarlaştırıcılar.*

[a] *ckuden@cu.edu.tr* *https://orcid.org/0000-0002-2663-9828*

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Introduction

Conventional methods for treating diseases like periapical infection and permanent pulpitis include root canal therapy. Three major objectives of root canal therapy are proper instrumentation, disinfection and obturation of root canal system. A smear layer and plugs of organic and inorganic tissue fragments of calcified tissue, as well as organic components like pulp tissue debris, odontoblastic processes, microorganisms, and blood cells, are produced by endodontic instrumentation in the dentinal tubules.[1] Because of its efficiency in destroying bacteria and dissolving organic tissue, sodium hypochlorite (NaOCl), a chemotherapeutic irrigation chemical, is recognized as the "gold standard" for root canal irrigation.[2] NaOCl, on the other hand, had a high toxic level for periradicular tissues and was unable to remove the smear layer. Moreover, it decreased radicular dentin microhardness.

It has been claimed that chelating or decalcifying solutions, such as ethylene diamine tetra acetic acid (EDTA), are effective at removing the smear layer.[3] However, it has been observed that these chemical substances modified the Calcium/Phosphorus (Ca/P) ratio of the dentin surface as well as the chemical nature of human dentin.[4] Changes in the Ca/P ratio could modify the proportion of organic and inorganic components, which would then alter the dentin's hardness characteristics.[5]

For both cleaning out root canals and eliminating the smear layer with a low-intensity laser, photodynamic treatment (PDT) stands out as an effective equivalent option.[2] It has been demonstrated that PDT has the potential to be just as successful as NaOCl in disinfecting root canals when comparing both of them in terms of the antibacterial efficiency in root canal therapy.[6,7] Methylene blue (MB) and toluidine blue O (TBO), two of the most popular and extensively studied phenothiazines (synthetic non-porphyrin chemicals) used in PDT, shown adequate antibacterial activity for root canal disinfection.[6,8,9] Curcumin (CUR), the primary ingredient in turmeric powder and another photosensitizer, has lately been employed in the PDT.[9,10] CUR was a potentially microbial-reducing anionic, polyphenolic, and lipophilic chemical that might act as a photosensitizer.[10-12]

The microhardness of root canal dentin was impacted by the irrigation and disinfection procedures,[13,14] and photosensitizers used in PDT may have an impact on the structure of radicular dentin by precipitating or changing the collagen matrix.[6,15-18] However, there is no knowledge in the literature the effect of photosensitizers on the radicular dentin microhardness after root canal disinfection with PDT. Therefore, the purpose of this *in vitro* study was to explore the effect of PDT using three different photosensitizers (MB, TBO and CUR) on the radicular dentin microhardness in comparison with NaOCl. The null hypotheses tested was the different photosensitizers had no influence on the the radicular dentin microhardness.

Material and Methods

This study was approved by the Çukurova University Faculty of Medicine Clinical Research Ethics Committee's report numbered 2020/105.53 Based on previous study,[19] a power analysis using G*Power 3.1 (Heinrich Heine University, Düsseldorf, Germany) showed that minimum sample size of 17 for each group provided a power of 80% to detect significant differences at a 0.05 significance level. Thus, 20 specimens for each group were used in the present study.

The root canal treatments of 100 human premolar teeth were performed by shaping with the Reciproc system (Reciproc; VDW, Munich, Germany). The study's inclusion criteria included teeth extracted for periodontal or orthodontic reasons that did not exhibit cracks, fractures, inclinations, or resorption along the root and had a single straight canal and apex. The periapical radiographs of the teeth were used to determine the canal diameter at the bucco-lingual and mesio-distal directions. Teeth having canal diameters greater than 1 mm were disqualified from the study. An ultrasonic scaler was used to remove adherent debris, plaque and periodontal ligament on teeth.

The working length of the teeth was determined by using a #10 K-file, keeping it 1mm shorter than the tooth apex. The R25 file (Reciproc; VDW, Munich, Germany) was used to prepare the root canals. After every three pecking motions, irrigation was performed and a total of 10 mm of distilled water was used and the root canals were dried by using sterile paper points (Dentsply Sirona, York, PA, USA). The specimens were randomly distributed according to the final disinfection method; NaOCl+EDTA, PDT with TBO, PDT with CUR, PDT with MB and distilled water (DS) (n=20).

Group NE: The root canals were irrigated with 5 ml of 2.5% NaOCl for 1 min, with 5 ml of 17% EDTA for 1 min and with distilled water for 1 min, respectively.

Group MB: The root canals were filled with MB (50 mg/l) and kept in the dark for 5 min before irradiation. A diode laser (SiroLaser Advance Plus; Dentsply Sirona, Bensheim, Germany) providing monochrome light at 660 nm wavelength was applied to the dentin surface with a fiber optic tip of 320 µm at a power of 100 mW for 90 s in continuous mode with helical movements in the apical-cervical direction.[16] Total energy dose of 9J was given to each canal. 9 J of total dose delivery and 320 J/cm2 of energy density.

Group CUR: The root canals were filled with CUR (500 mg/l) and kept in the dark for 5 min before irradiation. For the activation of curcumin, a fiber optic tip with a diameter of 300 µm and a blue LED light (λ 480 nm, Valo Cordless; Ultradent, South Jordan, UT, USA) were applied to the root canals with helical movements at 1000 mW/cm^2 standard power for 60 s.[13]

Group TBO: The root canals were filled with TBO (100 mg/l) and kept in the dark for 5 min before irradiation. The diode laser (660 nm, SiroLaser Advance Plus) was applied

to the dentin surface with a fiber optic tip of 320 µm at a power of 220 mW for 60 s in continuous mode with helical movements in the apical-cervical direction.[20] Total energy dose of 13.2J was given to each canal.

Group DS: No disinfection protocol was used.

The fiber optic tip was introduced into root canals to 2 mm short of the working length for all photosensitizers during the activating process. All photosensitizers were agitated for 1 min using an ultrasonic tip coupled to an ultrasonic unit (EMS, Nyon, Switzerland) avoiding contact with the root canal walls. All photosensitizers were withdrawn from the root canal with a syringe and the root canals were washed with distilled water after the disinfection process. The root canals were dried with paper points.

The grooves were prepared on the buccal and lingual surfaces of the prepared teeth, parallel to the long axis of the tooth, without touching the root canals. The roots were divided into two parts by means of a cement spatula placed in these grooves. The root canal dentin microhardness was evaluated by the Vickers test method. Three measurements were made by applying 300 g of force for 15 seconds and the average was calculated. It was recorded as the Vickers hardness value.

The data were analyzed by using the SPSS program for Mac version 26 (IBM SPSS Inc, Chicago, IL) The Shapiro-Wilk test was used to determine the normality distribution of the data assessed. The values of the PBS were analyzed by one-way ANOVA and Dunnett's post hoc tests. All data was evaluated with 95% of confidence interval (α=0.05).

Results

The values of the root canal microhardness for each root canal disinfection method were represented in Figure 1. The highest root canal dentin microhardness among the groups was observed in the CUR group. In disinfection with PDT, different photosensitizers did not show a significant difference in terms of root canal dentin microhardness. However, NE group showed significantly lower microhardness values than DS, TBO, CUR and MB groups (p=0.026, 0.01, 0.002 and 0.001 respectively).

Discussion

The disinfection of root canals is essential for the recovery of periapical pathologies and to prevent recurrence of endodontic diseases. This study evaluated the effect of MB, CUR and TBO photosensitizers used in PDT on the microhardness of radicular dentin.[21] There was no difference among the photosensitizer groups. However, conventional irrigation method, NaOCl/EDTA indicated significantly lower microhardness value than the other groups. Therefore, the H0 hypotheses were rejected.

By analyzing the plastic and elastic deformation of a substrate, hardness is one of the mechanical characteristics that may be utilized to evaluate the mineral changes in dentin.[22] The Vickers indenter method was employed in earlier research to evaluate the dentin's hardness.[23,24] Microhardness measurements have been shown to offer indirect evidence of mineral loss or growth in the tooth hard tissues.[24] Although the Knoop hardness test was employed for surface changes of dental hard tissues in some research, previous investigations have demonstrated the applicability and feasibility of the Vicker's microhardness test for evaluating surface changes of dental hard tissues treated with chemical agents.[1] In this research, it was shown that the Vicker's microhardness test identifies surface alterations following treatment with PDT and conventional disinfection methods.

The combination of NaOCl and EDTA is routinely used to dissolve inorganic and organic components in the smear layer of the radicular dentin.[18,25,26] The proteolytic NaOCl had a negative effect on the collagen structure of radicular dentin, correspondingly dentin microhardness.[1,27] It has been shown that EDTA binds calcium, leading to the dissolution of root dentin mineral components, and inactivation of alkaline phosphatases, which play an important role in the formation of mineralized tissue.[28] Also, it was known that the exposure time of EDTA in root canal decreased the microhardness of dentin.[1] Although the sequential use of these solutions may produce satisfying results for revealing dentin tubules and able to ease adhesion of root canal sealer,[29] it had detrimental influence dentin hardness.[30,31] The present the study was in parallel with those above studies.

PDT using with MB, CUR and TBO photosensitizers was approved antibacterial activity in the disinfection of root canals and has the ability to effectively destroy gram-negative and gram-positive bacteria.[8,12,32] Therefore, the present study investigated the effect of PDT disinfection using these photosensitizers on the radicular dentin microhardness. Regarding the limitation of PDT, the photosensitizer may remain on the dentin structure and act as a diffuse chemical layer into dentinal tubules.[33] Therefore, in this study, inorganic precipitates formed after PDT might have contributed to dentin hardness.

In addition, methylene and toluidine blue, which are cationic, may bind to anionic molecules such as phosphate in hydroxyapatite, thereby affecting the calcium/phosphate ratio.[19] This may have provided a higher hardness value than the control group. Hydrophilic materials exhibit high wettability and low contact angle for dentin substrate.[34] However, there was no difference between hydrophobic CUR and hydrophilic MB and TBO groups. This may be due to the use of curcumin in higher concentrations than other photosensitizers.

In a study examining the effects of different concentrations of CUR and MB on radicular dentin hardness, there was no difference between the control group and the MB group, while both 500mg/L and 1000mg/L concentrations of CUR showed lower hardness values compared to the control group.[19] In another study investigating the effect of different concentrations of MB and light activation on root canal microhardness, no difference was found compared to the control group.[35] These results are not compatible with our study and can

be attributed to the use of different hardness measurement methods.

The current study presents some inherent limitations due to the *in vitro* design of the study. Evaluating the physical effects of natural and synthetic photosensitizers on the radicular dentin together with other mechanical tests such as fracture resistance, elastic modulus may be helpful in understanding the further effects of these photosensitizers. Although it has been tried to be limited by various methods, substrate homogeneity or standardization should be considered due to the changing characteristics of the natural teeth used in the study. Moreover, the lack of consensus for factors such as the concentrations, irradiation times and power density of photosensitizers used may be shown as the limiting aspect of the PDT. The strength of this study was first that it simultaneously evaluated the effect of most used photosensitizer types on radicular dentin hardness.

Conclusion

The present study conducted reveals the effect of PDT using with different photosensitizers on dentin microhardness. PDT had a positive effect on root canal dentin microhardness compared to the traditional disinfection method.

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Conflict of interest

The authors declared no conflict of interest regarding the publication of this article and any of the materials used in this study.

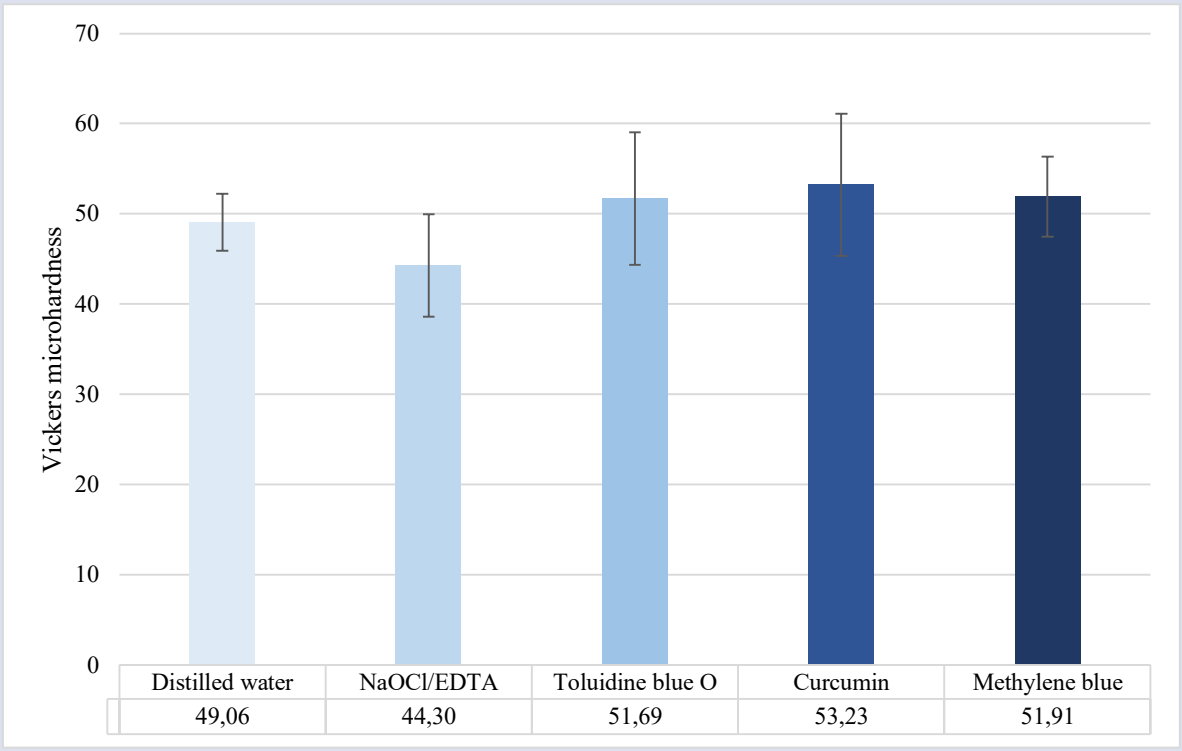

Figure 1. Vickers microhardness values of radicular dentin exposure to distilled water, NaOCl/EDTA, and PDT used with different photosensitizers

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