Effect of Morinda Citrifolia on the Microhardness of Root Canal Dentin

Morinda Citrifolia'nın Kök Kanal Dentin Sertliği Üzerine Etkisi

Hacer Deniz ARISU,[a]
Baran Can SAĞLAM,[a]
Evrim ELİGÜZELOĞLU,[b]
Mine Betül ÜÇTAŞLI[a]

[a]Department of Operative Dentistry and Endodontics,
Gazi University Faculty of Dentistry, Ankara
[b]Department of Operative Dentistry and Endodontics,
Yüzüncü Yıl University
Faculty of Dentistry, Van

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Yazışma Adresi/*Correspondence:*
Evrim ELİGÜZELOĞLU
Yüzüncüyıl University
Faculty of Dentistry,
Department of Operative Dentistry and Endodontics, Van,
TÜRKİYE/TURKEY
eeliguzeloglu@yahoo.com

ABSTRACT Objective: During root canal preparation, irrigation is recommended as a means of removing debris from the root canal, killing microorganisms, and dissolving necrotic and vital tissue remnants. For this reason, various irrigation solutions have been used in root canal treatment. The aim of this study was to evaluate the effects of 1% sodium hypochlorite (NaOCl), 6% Morinda citrifolia juice (MCJ), 2% chlorhexidine (CHX) solution or distilled water on the microhardness of root canal dentin. **Material and Methods:** The roots of 40 single-rooted mandibular premolar teeth were bisected longitudinally. Before and after irrigation, microhardness values were obtained for the cervical, middle and apical levels of the root canal from a 0.5 distance utilizing a Vickers microhardness tester with a 50 g load and 15 sec dwell time. The percent change in microhardness was calculated. The results were then analyzed through one-way ANOVA and Tukey post hoc tests. Comparisons with respect to root levels were analyzed with Friedman's test and the comparisons between before and after treatment values were made using the Wilcoxon test. **Results:** MCJ and NaOCl decreased the root canal dentin microhardness significantly ($p< 0.000$). CHX and distilled water, however, had no effect on root canal dentin microhardness ($p> 0.05$). **Conclusion:** This in vitro study showed that, the use of 6% MCJ as an irrigation solution affected the dentin microhardness.

Key Words: Root canal irrigants; chlorhexidine

ÖZET Amaç: Kök kanalının hazırlanması esnasında, kök kanalından debrisi uzaklaştırmak, mikroorganizmaları yok etmek, nekrotik ve vital doku kalıntılarını eritmek için irrigasyon önerilmektedir. Bu nedenle, kök kanalının tedavisinde çok sayıda irrigasyon solüsyonu kullanılmaktadır. Bu çalışmanın amacı, %1 sodyum hipoklorit NaOCI), %6 Morinda citrifolia suyu (MCJ), %2 klorheksidin (CHX) solüsyonu ve distile suyun kök kanalı dentin mikrosertliği üzerindeki etkisini belirlemektir. **Gereç ve Yöntemler:** Tek köklü mandibular premolar 40 adet dişin kökleri dikey olarak ortadan ikiye ayrıldı. Vickers mikrosertlik test cihazı kullanılarak, irrigasyondan önce ve sonra 50 g kuvvet ve 15 sn uygulama süresinde kök kanalının servikal, orta ve apikal seviyelerinde 0.5 mm uzaklıktan mikrosertlik değerleri ölçüldü. Mikrosertlikteki yüzde değişimi hesaplandı. Elde edilen veriler tek yönlü ANOVA ve Tukey post hoc testleri ile analiz edildi. Kök seviyeleri ile ilgili karşılaştırma Friedman testi, tedavi öncesi ve sonrası değerlerin karşılaştırılması ise Wilcoxon testi kullanılarak analiz edildi. **Bulgular:** MCJ ve NaOCI, kök kanalı dentin mikrosertliğini belirgin düzeyde azaltmıştır ($p< 0.000$). CHX ve distile su ise kök kanalı dentin sertliği üzerine etki etmemiştir ($p> 0.05$). **Sonuç:** Bu in vitro çalışma göstermiştir ki, %6'lık MCJ'nin irrigasyon solüsyonu olarak kullanımı mikrosertliği etkilemektedir.

Anahtar Kelimeler: Kök kanalı sulayıcıları; klorheksidin

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The success of root canal treatment depends on the thorough cleaning and three-dimensional shaping of the root canal system.

During root canal preparation, irrigation is recommended in order to remove debris from the root canal, kill microorganisms, and dissolve necrotic and vital tissue remnants. For this reason, various irrigation solutions, including sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), chlorhexidine gluconate (CHX), and ethylene diamine tetra acidic acid (EDTA) solutions have been used in root canal treatment.

It is a well-known fact that many infectious diseases have been treated with herbal remedies throughout humankind's history. For over 2000 years, Polynesians have used the juice of the exotic Morinda citrifolia fruit or the noni plant, in folk remedies for its antibacterial, antiviral, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects.[1-3] Murray et al. found that 6% (the minimum inhibitory concentration on *Enterococcus faecalis*) Morinda citrifolia juice (MCJ) was as effective as NaOCl and EDTA for the removal of smear layer, and they concluded that MCJ could be a potential root canal irrigation solution.[4]

Hardness is a measure of a material's resistance to localized plastic deformation, in other words, the ability of a material to resist a permanent indentation.[5] The measurement of the hardness of a material is one of the simplest nondestructive mechanical characterization methods. Hardness tests provide a numerical value allowing for a distinction between materials submitted to the penetration of a specific intender. The values obtained depend upon several factors, such as the Young's modulus of the material, and the yield stress in compression anisotropy, among others. Thus, the hardness value cannot be considered a basic property of the material, but rather an indicator of its behaviour, given the specific conditions of the penetration test. In a conventional Vickers hardness test, a sample is indented with a pyramidal probe with load Q. The visualization of the indentation mark allows for the measurement of its diagonals and thus, the determination of the Vickers hardness number.[6]

Irrigation solutions used in chemomechanical debridement of the root canal system might yield changes in the chemical and physical properties of root canal dentin,[7,8] In previous studies, it has been reported that some chemical endodontic irrigation solutions are capable of causing alterations in the chemical composition of dentin.[7-9] Any change in the Ca/P ratio may change the microhardness, permeability, and solubility characteristics of dentin.[10] It was reported that different concentrations of EDTA, EDTAC, and ethyleneglycotetraacetic acid (EGTA) are capable of decreasing the microhardness of root canal dentin.[6,11] These mineral changes in the content of dentin could also affect negatively the sealing ability and adhesion of resin-based root canal sealers to root canal dentin.[12,13] Although there are a number of studies that reported the effect of different chemical solutions on the microhardness of root canal dentin, the mechanical effects of MJC on root canal dentin is still unknown.[6-9,11]

The aim of this study was to evaluate the effects of 1% NaOCl, 6% MCJ, 2% CHX solution, or distilled water on the microhardness of root canal dentin. The null hypothesis tested is 6% MCJ when used as an endodontic irrigant does not change the microhardness of root canal dentin.

MATERIAL AND METHODS

Forty single-rooted mandibular premolar teeth extracted for orthodontic reasons were collected and were stored in distilled water at 4°C for a maximum of one month. The crowns were removed at the cemento-enamel junction and the roots were bisected with a low speed saw (Mecatome T201A, Pressi, France) longitudinally to obtain 80 root halves. The specimens were embedded in acrylic resin, leaving the root canal dentin exposed and the dentin surfaces were ground on a circular grinding machine with 300, 400, 600, 800, 1000 and 1200-grit SiC abrasive papers, respectively, under running water to obtain a flat surface. The Vickers hardness values were measured using an HMV Microhardness Tester (Shimadzu, Tokyo, Japan). Before application of the tested endodontic irrigation solutions, 3 separate indentations, each using 50 g load and

15 seconds dwell time, were made along the 0.5 mm distance to the root canal wall at the apical, midroot and cervical levels. Then, the specimens were treated with 1% NaOCl, 6% MCJ, 2% CHX solution, or distilled water (negative control) for 15 minutes in a magnetic stirrer bath containing 20 mL of the tested solution. Post-treatment indentations were made on each specimen adjacent to the initial indentations, in the same manner and the microhardness values were recorded. For each specimen, the percent change in microhardness was calculated using the following formula:

[(Initial microhardness-post-treatment microhardness)/Initial microhardness] X 100

The results were then analyzed using one-way ANOVA and Tukey post hoc tests. Comparisons within each group with respect to root levels were analyzed with Friedman's test and the comparisons between the microhardness values before and after treatment were made with the Wilcoxon test.

RESULTS

The percent change in microhardness values of the entire root canal dentin (apical, middle and coronal regions) and changes in microhardness values with respect to the apical, middle and coronal regions were represented in Table 1.

While MCJ and NaOCl decreased the root canal dentin microhardness significantly ($p< 0.000$), CHX and distilled water had no effect on root canal dentin microhardness ($p> 0.05$).

MCJ decreased the overall root canal dentin microhardness significantly more than the other test solutions did (Figure 1).

There was no significant difference between chlorhexidine and distilled water with respect to the percent change in the microhardness of the entire root canal dentin ($p= 0.917$).

In all treatment regions, the same statistical ranking was obtained for percent change in dentin microhardness ($p= 0.05$).

The Friedman test showed that for all tested solutions, there was no statistically significant difference in percent change in microhardness with respect to the application regions (distilled water 0.497, MCJ 0.061, NaOCl 0.202 and CHX 0.459).

The Wilcoxon test showed that there were statistically significant differences in all groups and in all regions between the before and after treatment microhardness values ($p< 0.05$).

DISCUSSION

The importance of irrigating the root canal with an antimicrobial irrigant during preparation to remove microorganisms and their toxic metabolic products has been recognized for over 60 years.[14] In infected root canals, the smear layer produced

TABLE 1: The mean percent changes in microhardness with respect to coronal, middle, apical and total (coronal+middle+apical) root canal dentin following treatment with the test solutions.

		Distilled Water	MCJ	NaOCl	CHX
Coronal	Mean±SD %	2.97 ± 2.76	33.48 ± 9.47	17.72 ± 7.06	4.10 ± 2.16
	Min %	0.30	22.42	7.53	0.40
	Max %	9.57	46.76	29.05	7.56
Middle	Mean±SD %	2.64 ± 2.82	30.88 ± 9.24	12.46 ± 5.96	3.82 ± 2.45
	Min %	0.10	19.50	3.31	0.37
	Max %	9.33	43.94	20.57	7.40
Apical	Mean±SD %	2.47 ±1.40	26.62 ± 6.01	15.86 ± 7.74	3.18 ± 1.47
	Min %	0.46	18.23	11.41	1.37
	Max %	4.57	34.73	36.96	5.07
Total	Mean±SD %	2.69 ± 2.34	30.33 ± 8.59	15.35 ± 7.07	3.70 ± 2.03
	Min %	.10	18.23	3.31	.37
	Max %	9.57	46.76	36.96	7.56

CHX, Chlorhexidine; MCJ, Morinda citrifolic juice; NaOCl, Sodium hypochlorite; SD, standard deviation.

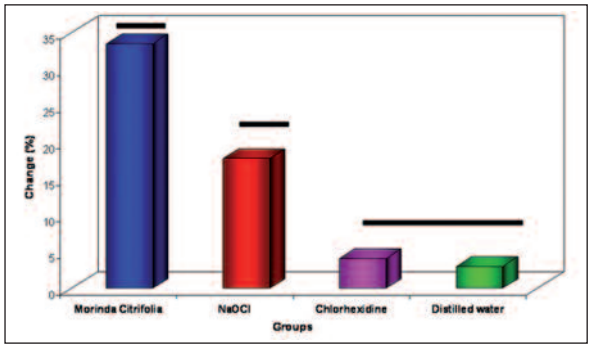

FIGURE 1: The percent changes in microhardness and homogeneous subsets for α= 0.05 of the entire root canal dentin (apical, middle, and coronal regions) in groups.

by instrumentation should be removed, because bacteria may have invaded dentinal tubules and accessory canals. The smear plugs produced during instrumentation should be removed in order to facilitate the antibacterial effect of the intracanal medicaments.[15] The smear layer constitutes a negative influence on the sealing ability of obturated canals, since it is a porous and weakly adherent interface between the filling and the dentine wall.[16]

Determining the microhardness can provide indirect evidence of mineral loss or gain in the dental hard tissues.[17] Panighi and G'Sell reported a positive correlation between hardness and the mineral content of the tooth.[18] Marending et al. demonstrated that with the dissolution of organic dentine components, there is a concentration-dependent hypochlorite effect on the mechanical dentine properties.[19]

Dentin hardness is related to location and its value decreases as the indentations tested are made closer to the pulp.[20,21] Pashley et al. reported an inverse correlation between dentin microhardness and tubular density.[22] The histological pattern of the root canal dentin probably contributes to hardness reduction at the pulpal interface. The increased number of widely opened dentin tubules that were free of peritubular dentin near the pulp offered little resistance to the testing intender.[23] Since the irrigation solutions affect the dentin walls more strongly clinically, in the present study the measurements were carried out at a 0.5 mm level from the root canal spaces, as Erdemir et al. suggested for standardization.[24]

The relative softening effect on dentinal walls exerted by chemical irrigants could be of clinical benefit, since it permits rapid preparation and facilitates negotiation of small tight canals, but these alterations also affect the sealing ability and adhesion of sealers to treated dentin surfaces.[12,13,25] Oliveira et al. studied the effect of endodontic irrigation solutions (1% NaOCl, 2% CHX) on the microhardness of root canal dentin and reported that irrigation with NaOCl or CHX significantly reduced the microhardness of root canal dentin.[7] In the present study, 1% NaOCl and 6% MCJ significantly reduced the microhardness values. So the null hypothesis of the current study was rejected. However, 2% CHX had no effect on the surface microhardness of root canal dentin. Ari et al reported that irrigating the root canal dentin with 5.25% NaOCl, 2.5% NaOCl, 17% EDTA, and 3% H_2O_2 caused a significant reduction in microhardness, but that 0.2% CHX had no softening effect on the root canal dentin.[8] The differences between these results may arise from the differences in their methodology and from the fact that dentin is not a homogeneous material.

The use of MCJ as an endodontic irrigant might be of interest to patients and endodontic professionals in terms of the growing trend to seek natural remedies as part of dental treatment.[26] Murray et al. showed that 6% MCJ was as effective as 6% NaOCl as an irrigation solution, when used with a final flush of EDTA.[4] They concluded that MCJ could be a possible alternative to NaOCl as an intracanal irrigant. In addition, they determined that the minimum inhibitory concentration of MCJ on *E. faecalis* growth in test tubes was the 6% solution. Depending on these results, a 6% concentration of the MCJ solution was used as irrigation solution in the current study. Kandaswamy et al. investigated the antimicrobial activity of 2% chlorhexidine gel, propolis, MCJ, 2% povidone Iodine (POV-I), and calcium hydroxide on *E. faecalis*-infected root canal dentine at two different depths (200 mL and 400 mL) and three time intervals and they concluded that Propolis and MCJ were effective against *E. faecalis* in dentine of extracted teeth.[27] Ring et al. evaluated the cytotoxic-

ity and to compare the effect of ten different endodontic irrigation and chelating treatments on dental pulp stem cell (DPSC) attachment to root canal surfaces and results of their study showed that MCJ/EDTA were among the most optimal of the irrigating solutions to help maintain the survival and attachment of DPSCs to be used as part of the regenerative endodontic treatment.[28]

However, in the literature, there is no report on the mechanical effects of MCJ on root canal dentin. Changes in the mineral content of superficial dentin may adversly affect the sealing ability and adhesion of dental materials.[29] Further research is needed to determine the specific effects of MCJ irrigation on the sealing ability and adhesion of dental materials such as resin-based cements and root canal sealers, to dentin.

CONCLUSION

Within the limitations of this in vitro study, it may be concluded that:

- 2% CHX gluconate did not change the root canal dentin microhardness.
- 1% NaOCl and 6% MCJ significantly reduced the dentin microhardness values.

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