EUROPEAN ENDODONTIC JOURNAL

ORIGINAL ARTICLE

Diabetes Mellitus Affects the Microhardness of Root Dentine: An *in-vitro* Study

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ABSTRACT

Objective: This study was undertaken to compare microhardness and erosion susceptibility of root dentine in teeth extracted from diabetic and non-diabetic donors after the application of different root canal irrigants.

Methods: Forty-eight single-rooted premolars with single canals (24 each from diabetic and non-diabetic) were selected, and root canals were shaped by using rotary ProTaper files. Dentine slices of 4 mm were transversely sectioned from the middle root third. Specimens were assigned to four subgroups (n=6) and irrigated for 5 minutes: 1) 2.6% sodium hypochlorite (NaOCl); 2) 17% ethylenediaminetetraacetic acid (EDTA); 3) 2% chlorhexidine (CHX); and 4) normal saline. Surface microhardness was determined at 100- and 500-µm depths from the pulp–dentine interface. Scanning electron microscope (SEM) was used to determine the severity of dentine erosion. Data were analyzed by using two-way ANOVA, Post-hoc Tukey's, and Chi-square tests (P<0.05).

Results: Diabetes as well as NaOCl and EDTA decreased surface microhardness of dentine significantly (P<0.05). Diabetes had little effect on the erosion susceptibility of dentine (P>0.05).

Conclusion: Root canal irrigants can significantly lower the microhardness; specifically, in diabetic patients, and may be a factor affecting the longevity of root canal-treated teeth.

Keywords: Diabetes mellitus, erosion, microhardness, root canal irrigant

Please cite this article as: Saghiri MA, Rahmani B, Conte M, Nath D, Peters OA, Morgano SM. Diabetes Mellitus affects the microhardness of root dentine: An *in-vitro* study. Eur Endod J 2022; 7: 122-8

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Received 14 September 2021, Accepted 03 March 2022

Published online: 15 June 2022
DOI 10.14744/eej.2022.37029

HIGHLIGHTS

- Dentin makes up approximately 80% of the tooth, suggesting the need to explore its mechanical properties that are adversely affected by diabetes.
- The microhardness of dentin in diabetic samples was significantly lower than that in non-diabetic ones.
- NaOCl and EDTA had more deleterious effects on the root canal dentin in diabetic samples.
- Erosion susceptibility of the dentin is less affected by diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome caused by impaired insulin secretion from pancreatic beta cells and/or impaired insulin function in peripheral tissues (1, 2). According to the CDC, in 2018, more than 34 million Americans, or 10.5% of the population, had diabetes (3).The International Diabetes Federation (IDF) estimated that more than 451 million adults (1 in 11 adults) had diabetes mellitus globally in 2017 (4). DM has been regarded as a disease modifier in the oral cavity, significantly altering the physiochemical properties of dentine (5, 6). The damage of DM to the tooth enamel is also a relevant clinical concern, but enamel can be affected by many other exogenous factors such as diet, therefore, the focus is concerned with dentine's biomechanical features (7). Physiologically and mechanically, dentine is a complex structure that makes up approximately 80% of the tooth volume compared with enamel, which only contributes 20% (8). The large tissue volume of dentine, consisting of an inorganic and organic matrix, provides robust mechanical support to the tooth. However, the surface area of dentine is often invaded by pathological diabetic conditions, resulting in subsequent root canal therapy (Fig. 1). Additionally, this metabolic disorder affects the outcome of endodontic procedures, suggesting that diabetes is a modulating factor for endodontic infections (9). There is an increased prevalence of endodontically treated teeth with periradicular lesions, and there is a disproportionately high percentage of clinically severe pulpal infections in diabetic patients, suggesting that there could be

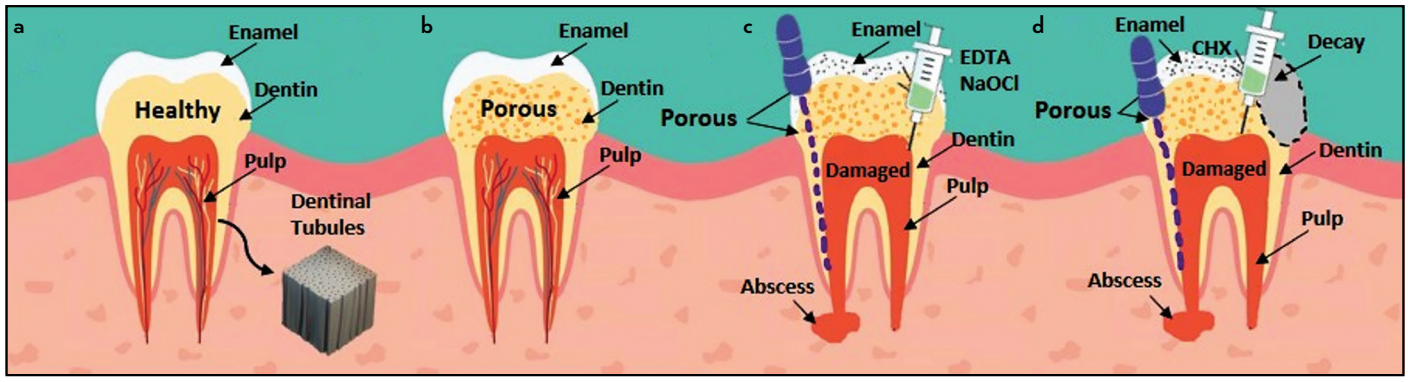

Figure 1. Tooth structure loss resulting from diabetes mellitus. (a) Healthy tooth with dentine's unique microstructure. (b) Demineralized dentine as a result of endogenous factors. (c) Demineralized enamel as a result of exogenous factors and formulation of painful abscess from damaged pulp. Root canal therapy along with endodontic irrigants (EDTA, NaOCl) was exhibited, further contributing to reduced microharness. (d) Completely destroyed tooth as evident by dental caries. Root canal therapy along with irrigation using CHX was performed, resulting in minimal damage on surface microhardness

EDTA: Ethylenediaminetetraacetic acid, NaOCl: Sodium hypochlorite, CHX: Chlorhexidine

some differences in the natural history of endodontic diseases in these patients (9). Some alterations can be seen in the dental pulp in diabetic patients, such as the circadian rhythms of pulp sensitivity (10). There is less likelihood of success in cases with preoperative periradicular lesions and compromised healing of periapical lesions and retention of endodontically treated teeth, with an increased incidence of retreatment (9, 11).

Mechanical instrumentation of the root canal produces debris and a smear layer consisting of organic and inorganic components; therefore, it is customary to use irrigation solutions for rendering successful root canal treatment (12). Although root canal irrigants have several advantages, such as flushing out the debris, disinfection, removal of the smear layer, and lubrication of the canal walls (13), they might negatively affect physical properties of dentine such as microhardness, making the dentine more susceptible to erosion (14) by altering the calcium-to-phosphorus ratio of the dentine surface (15). Any changes in the mineral contents of dentine can be observed indirectly through microhardness evaluations (16). Sodium hypochlorite (NaOCl) (14, 17), and 17% ethylenediaminetetraacetic acid (EDTA) (14) can negatively affect microhardness while 2% chlorhexidine (CHX) has minimal effects on this parameter (18). The penetration of root canal irrigants into the dentinal tubules appears to play an important role in altering microhardness, while erosion from to the use of irrigants is less important in this regard (18).

From a mineralogical perspective, it is expected that the crystalline parameters of dentine may also be affected by diabetes (19). EDAX (energy dispersive X-ray spectroscopy) analysis of root dentine of diabetic specimens revealed similar elements to non-diabetic specimens except for strontium which was absent in the diabetic specimens (20, 21). It is noteworthy to mention that strontium affects the crystallinity of hydroxyapatite in bone (22), which puts this element, along with calcium, as the first suspect in the alteration of the physical properties of dentine. Additionally, diabetic patients showed higher susceptibility to instrumentation and also had reduced shear bond strength (23, 24). Currently, root canal treatments are carried out in the same manner for diabetic patients and non-diabetic patients, apart from the difficulty of locating the root canal orifices because of the presence of pulpal calcifications, or canal negotiation because of the higher calcification rate of radicular pulp (25-27). However, the reason for the lower success rate of endodontic treatment is still unknown. There is insufficient information available on the physical properties of root dentine in diabetic patients, in relation to the use of common root canal irrigants. Therefore, this study was designed to investigate the effects of diabetes on the microhardness of root dentine subsequent to the use of different root canal irrigants and to evaluate the erosion susceptibility of dentine with these irrigants. The null hypothesis for this study was that dentinal microhardness is not affected by diabetes or different irrigations used.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board (IRB) at the Faculty of Dentistry, Rutgers School of Dental Medicine, New Jersey. Forty-eight single-rooted maxillary and mandibular premolars with single root canals were used in this study, based on the inclusion and exclusion criteria mentioned below. The teeth were extracted for periodontal or orthodontic reasons. Half of the teeth were collected from diabetic patients or donors. The teeth were stored in 0.5% chloramine-T at 4°C for 15 days before use, for disinfection, followed by storage in distilled water until used (28).

Inclusion and exclusion criteria

- Diabetic patients with no other systemic diseases (verbally declared by the patients)
- Patients had diabetes mellitus type II for 5 to 15 years, and the condition should be controlled for at least one year.
- Patients under 20 and over 60 years of age were excluded.
- Teeth with any cracks, defects, or cervical caries (confirmed by using stereomicroscope), along with those stored in antibacterial or fixative solutions, and those with previous root canal treatment were excluded.

Specimen size calculation

Power analysis (PA) was performed before the statistical analysis. The power was 80%, the margin of error was 5%, and an

effect size of 0.62, which was based on a pilot study conducted by the research team and similar previously reported studies by Dikmen et al. (24) and Saghiri et al. (28). The specimen size was selected to be six in each subgroup (n=6).

Specimen preparation

The study protocol was similar to a previous study (29). Briefly, 48 specimens were prepared and assigned to two groups [diabetic (n=24) and non-diabetic (n=24)]. The teeth were decoronated, and the root canals were prepared with ProTaper rotary instruments (Dentsply-Maillefer, Ballaigues, Switzerland) by using Sx, S1, S2, F1, F2, F3, and F4 to apical size of #40, one mm short of the apex. The working length was established 1-mm short of the length at which a #10 K-file (Dentsply-Maillefer, Ballaigues, Switzerland) exited the apical foramen. Final preparation was carried out with F3 (tip size=30). Copious irrigation was performed during instrumentation with 2.6% NaOCl (5 mL). The final flush of the irrigation procedure was performed with 5 mL of normal saline solution (0.9% sodium chloride irrigation). A horizontal 4-mm-thick dentine disk was obtained from the mid root region by using a low-speed saw (Isomet; Buehler Ltd., Lake Bluff, NY) with a diamond disc under continuous water irrigation.

Treatment procedure

All the specimens from both the diabetic and non-diabetic groups were randomly assigned to four subgroups (n=6) in terms of the irrigant used. The lumen of each section was occluded with adhesive wax at the lower surface of the disk and filled with the following irrigants, being refreshed once per minute for a total of 5 minutes:

- Subgroup 1: 2.6% NaOCl
- Subgroup 2: 17% EDTA (Pulpdent Corp, Watertown, MA)
- Subgroup 3: 2% CHX (Consepsis, Ultradent, UT)
- Subgroup 4: NSS (control group).

To prevent prolonged effects of the irrigants, the specimens received a final flush of 10 mL of distilled water immediately after the treatment.

Vickers hardness tests

This part of the study was similar to a previous study (18). Briefly, to perform the Vickers hardness test, a Micro Met 5100 durimeter microhardness tester (Buehler Ltd, Lake Bluff, IL) was used. The procedure was repeated three times and the mean numeric value of hardness was recorded as the hardness value for each specimen. In each specimen, three separate indentations were made at 100- and 500-µm depths from the pulp–dentine interface by using a 300-g load with a dwell time of 20 seconds at each measurement. The indentations were evaluated under an optical microscope, and the average length of their two diagonal lengths was used to determine the microhardness value (MHV). The average value of the results for the three indentations was considered as the representative hardness value for each depth.

Erosion

All the specimens were split longitudinally by using a cylindrical carbide bur (D&Z, Lemgo, Germany) in a high-speed handpiece under copious water spray without entering the canal. One-half of each specimen was selected randomly and prepared for SEM analysis at 20 kV, by using S-2500 Scanning Electron Microscope (SEM) (Hitachi, Pleasanton, CA). Briefly, the specimens were immersed in 2% glutaraldehyde for 24 h, rinsed three times with a sodium cacodylate buffer solution (0.1 M, pH=7.2), incubated in osmium tetroxide for 1 h, and dehydrated with increasing concentrations of ethyl alcohol (30–100%). Subsequently, they were placed in a desiccator for 24 h, mounted on a metallic stub, coated with 10 nm of gold, and evaluated at three pre-determined areas under an SEM (Hitachi, Pleasanton, CA) (×3000 magnification). Digital images were recorded (Microsoft Picture Manager, Redmond, WA) and standardized at 480×666 pixels. Two investigators scored the degree of dentinal tubule erosion blindly by considering the criteria proposed by Torabinejad et al. (30):

1. No erosion. All the tubules looked normal in appearance and size.
2. Moderate erosion. The peritubular dentine was eroded.
3. Severe erosion. The intertubular dentine was destroyed, with connections between the tubules.

A third investigator was asked to score the specimens in case of disagreement between the investigators.

Statistical analysis

Data were presented as means±SD and N (%). To analyze the effect of treatment type on microhardness in two groups, two-way ANOVA and Post-hoc Tukey's test were used. The comparison between qualitative variables (degree of erosion and patients' groups) was performed with chi-squared tests. The normal distribution was confirmed by using the Kolmogorov–Smirnov test. All the statistical analyses were conducted by using SPSS for Windows 25.0 (SPSS, Chicago, IL). Statistical significance was pre-set at P=0.05.

RESULTS

Microhardness

The means and standard deviations of microhardness values at depths of 100 µm and 500 µm are shown in Table 1. Based on the results of two-way ANOVA test, both factors, patient and treatment groups, showed a significant effect (P<0.05). All of the treatments decreased the microhardness significantly compared to NSS in both the diabetic and non-diabetic groups. There was a significant (P<0.05) difference observed between the subgroups of the two groups (diabetic and non-diabetic) as well.

Results demonstrated a significantly lower microhardness at 100 and 500 µm of teeth in diabetics compared with non-diabetics (P<0.05). Post hoc Tukey's test indicated that the mean values of microhardness were significantly lower for diabetic patients in all the treatment groups (Fig. 1a, b). Based on the mean values,

TABLE 1. Comparison of means and standard deviations of microhardness values at 100 and 500 µm between diabetic and non-diabetic patients in treatment groups*

Microhardness	Treatment patient	CHX 2%	Significance	EDTA	Significance	NaOCl	Significance	Saline	Significance
100 µm	Diabetic	22.02±5.59	***	17.83±4.1	***	24.05±5.75	***	37.27±4.58	***
	Non-diabetic	40.03±4.81		38±3.47		39.75±2.35		48.92±3.7	
500 µm	Diabetic	35.95±3.09	***	25.33±4.2	***	29.18±1.8	***	40.62±3.47	***
	Non-diabetic	49.95±3.24		38.6±1.74		41.76±3.76		51.67±3.4	

*: Post-hoc Tukey test, ***: Comparisons significant at the 0.05 level indicated by. CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid, NaOCl: Sodium hypochlorite

EDTA seemed to have reduced the microhardness the most in both diabetic and non-diabetic specimens (Fig. 2c, d).

Erosion

Chi-square tests demonstrated an association between the type of patient (diabetic and non-diabetic) and degree of erosion in different treatment groups (Table 2). Although severe dentine erosion in diabetic patients appeared to be more frequent after using CHX, EDTA, and NaOCl, with EDTA showing higher erosion (Table 2), the difference was not statistically significant (P>0.05).

DISCUSSION

This study shows differential effects of common endodontic irrigants for dentine harvested from diabetic subjects compared to normal controls. Therefore, the null hypothesis was rejected; specifically, the comparison between diabetic and non-diabetic groups showed that the microhardness values in diabetic specimens were remarkably lower than those in non-diabetic subjects.

There was a direct relationship between microhardness and location (distance from root canal lumen) of the evaluation, which might be because of the amount of calcified matrix per mm^2 of dentine (31). Moving away from the dentine–pulp interface, the amount of calcified matrix and peritubular dentine increases with less tubular density (31). The significantly lower microhardness of diabetic specimens might be the result of altered mineral contents in the dentine of these teeth (16, 26, 27) and higher tubular density (21). Microhardness evaluation provides information on dentine surface resistance. To eliminate the effect of testing variables, the loads applied to the specimens were similar. However, the microhardness values may be affected by the strain hardening and viscoelasticity of the materials tested (32, 33).

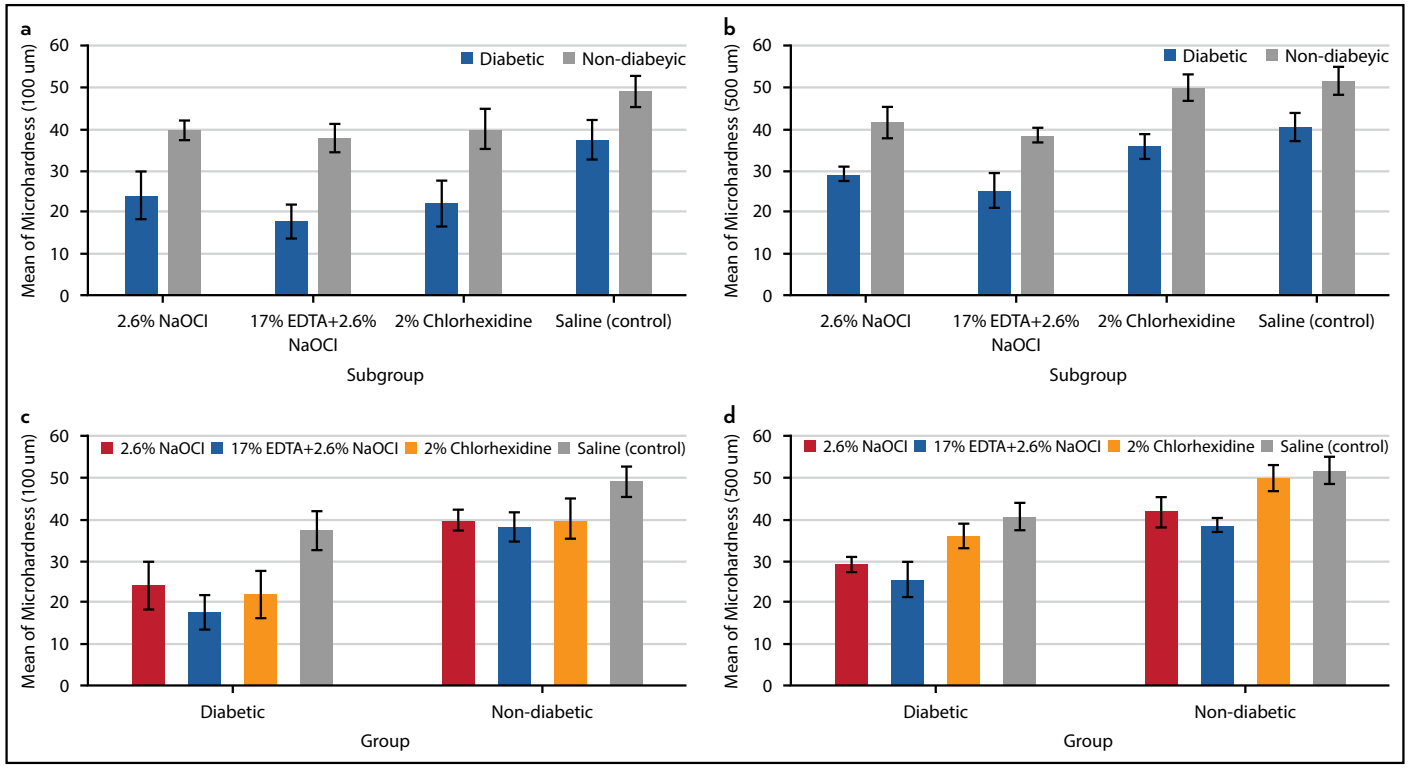

Figure 2. Microhardness values. (a) The mean and standard deviation of microhardness based on subgroups at depth of 100 µm; (b) The means and standard deviation of microhardness based on subgroups at depth of 500 µm; (c) The means and standard deviation of microhardness based on the groups (diabetic and non-diabetic) at depth of 100 µm; (d) The means and standard deviation of microhardness based on the groups (diabetic and non-diabetic) at depth of 500 µm

NaOCl: Sodium hypochlorite, EDTA: Ethylenediaminetetraacetic acid

TABLE 2. Erosion (n, %) frequency distributions for patients in different treatment groups

Treatment	Patient type	Erosion						P*
		No Erosion		Moderate		Severe		
		n	%	n	%	n	%	
CHX 2%	Diabetic	0	0	2	33.3	4	66.7	**0.588**
	Non-Diabetic	0	0	3	50	3	50	
EDTA								
	Diabetic	0	0	3	50	3	50	**0.588**
	Non-Diabetic	0	0	4	66.7	2	33.3	
NaOCl								
	Diabetic	0	0	3	50	3	50	**0.105**
	Non-Diabetic	1	16.67	5	83.30	0	0	
Saline								
	Diabetic	4	66.7	2	33.3	0	0	**0.27**
	Non-Diabetic	5	100	0	0	0	0	

*: Exact Chi-Square. CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid, NaOCl: Sodium hypochlorite

To omit the irrigation time duration variable, all root canal irrigants were used for 5 minutes in this study. These irrigants are among the most widely used irrigants (34, 35). The Vickers hardness test has been shown to be a suitable and reliable method for evaluating the microhardness and surface changes of the dentine (12, 15).

It has been reported that bacteria can penetrate the dentinal tubules up to 400–500 µm (36), while irrigants are able to penetrate up to 130 µm from the dentine–pulp interface (37); therefore, 100 and 500 µm were selected for evaluations in this study instead of the intact surface of the dentine. NaOCl decreased dentine surface microhardness in this study, consistent with a previous study (17). After 5 minutes of irrigation, 2.6% NaOCl decreased the dentine microhardness at both depths, which might be a result of the capability of NaOCl to dissolve approximately 17% of the weight of the organic component of dentine (38). EDTA, a commonly used chelating agent, decalcifies the inorganic portion of dentine and dissolves its mineral content (39); therefore, it renders the dentine less resistant to deformation (40).

The tubular density and push-out bond strength of MTA to dentine were affected in diabetic patients. Higher tubular density with less peritubular dentine and lower push-out bond strength of MTA to dentine in diabetic patients was reported (21). The SEM micrographs of the diabetic and non-diabetic specimens showed increased tubular density in diabetic specimens, consistent with a previous study (Fig. 3a, b). Considering the tubular density and tubules' diameter, it is expected that

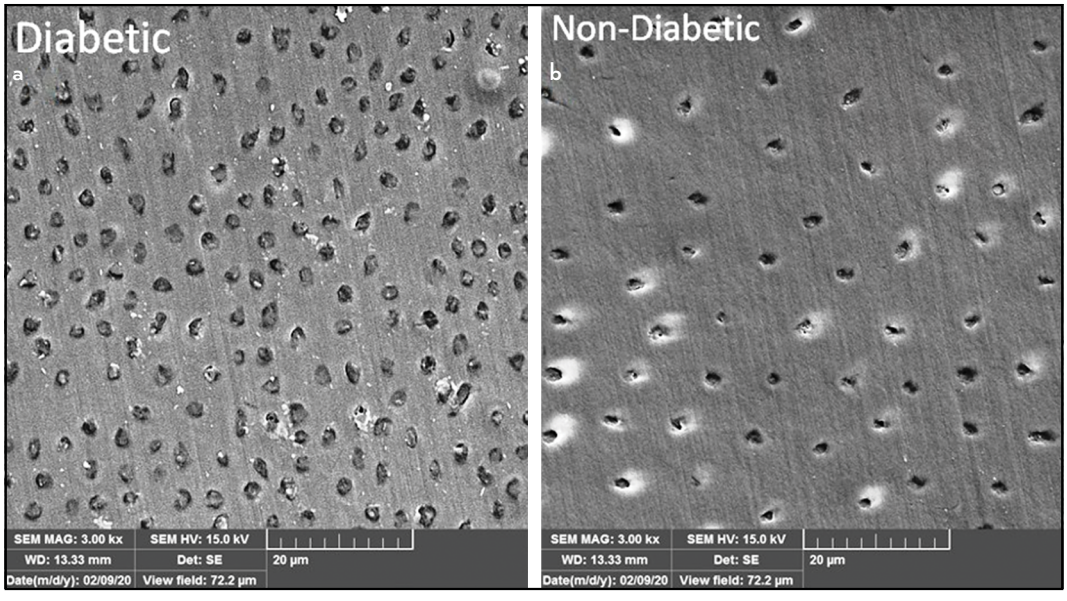

Figure 3. SEM micrographs of EDTA subgroups. (a) Dentinal tubule density of diabetic specimen at ×3000 magnification; (b) Dentinal tubule density of non-diabetic specimen at ×3000 magnification

SEM: Scanning electron microscope, EDTA: Ethylenediaminetetraacetic acid

the microhardness will be affected in the diabetic specimens. Furthermore, it is expected that the penetration and diffusion of the irrigation solution into the dentinal tubules will be substantial in diabetic specimens when compared to non-diabetic ones; therefore, it is anticipated that the irrigation solutions will affect microhardness more.

EDTA, compared with other irrigants, had more significant detrimental effects on dentine in diabetic specimens. Although 2% CHX decreased dentine microhardness at both depths in comparison with NSS, the difference was not significant, which might be because of the substantial effect of CHX (41) on the chemomechanical properties of dentine. Furthermore, CHX is unable to dissolve any necrotic tissues or smear layer which mechanically obstructs the dentinal tubules (42), resulting in limited penetration of the irrigant with an impact on microhardness.

Although evaluation of SEM micrographs revealed that the irrigants resulted in more severe erosion of the root dentine in diabetic specimens, the difference was not significant statistically. The erosion findings coincided with a previous study (43).

The middle third of the root specimens were selected because this part is less frequently affected by sclerotic dentine; at a lower rate, dentine sclerosis can make the evaluation of the erosion more difficult (44). Extensive erosion can be a contributing factor in making the root more susceptible to root fracture (45). Instrumented dentine specimens were used instead of standard specimens to mimic the clinical situation.

This study has some limitations; the duration of diabetes reported by the patients was not extremely accurate as the patients only verbally confirmed that they were diabetic for more than 5 years. In addition, most of the patients whose extracted teeth were included in this study were unable to do the blood work every six months as required. Hence, there was no established data or history regarding their fasting blood glucose and glycated hemoglobin (Hemoglobin A1C). Furthermore, systemic complications increase as the duration and level of high glucose amount increase (46). Although dentine may not be a perfect substrate for microhardness testing (47), it was chosen as it is the only hard tissue of the tooth which is formed by deposition during the lifetime and may be affected by diabetes. Age and sex are other factors that can affect the dentine microhardness that should be adjusted in these types of studies (7, 48).

It seems that the data presented could lead to consideration of strategy modification for diabetic patients to lower the detrimental effects of routine root canal treatment where the failure rate of such treatment is higher in these patients compared with healthy subjects. Diabetes and some commonly used root canal irrigants may play a role in lowering the success rate and longevity of endodontic treatment in diabetic patients. This strategy should consider measures to improve the physical properties, such as microhardness. Future studies should focus on other physical properties of dentine in these patients as the physical properties of the dentine are derived from both organic and inorganic components. Furthermore, molecular changes of the dentine in diabetic patients should be studied to better understand the underlying mechanisms that affect the physical properties of the dentine.

CONCLUSION

Under the limitations of this study, a history of diabetes along with commonly used root canal irrigants may significantly lower the dentine microhardness to a critical level. It is advisable to limit or avoid the use of chelating agents, such as EDTA, in patients with diabetes.

Disclosures

Acknowledgments: MAS is a recipient of the New Jersey Health Foundation Award. This publication is dedicated to the memory of Dr. H. Afsar Lajevardi, a legendary Pediatrician (1953-2015) (49). We will never forget Dr. H Afsar Lajevardi's kindness and support. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the affiliated organizations. The authors hereby announced that they have active cooperation in this scientific study and preparation of the present manuscript. The authors confirm that they have no financial involvement with any commercial company or organization with direct financial interest regarding the materials used in this study. Special thanks to Dr. Amir Fakhrzadeh to help in collecting the teeth for use in this current study.

Conflict of interest: The authors deny any conflict of interest.

Ethics Committee Approval: This study was approved by The Newark Health Sciences Institutional Review Board (Date: 21/03/2022, Number: Pro2021000043).

Peer-review: Externally peer-reviewed.

Financial Disclosure: This study did not receive any financial support.

Authorship contributions: Concept – M.A.S.; Design – M.A.S., B.R.; Supervision – M.A.S.; Funding - S.M.; Materials - M.A.S., S.M.M.; Data collection and/or processing – M.A.S., B.R., D.N.; Analysis and/or interpretation – M.A.S., B.R., M.C., D.N.; Literature search – M.A.S., B.R., D.N.; Writing – M.A.S., B.R., D.N.; Critical Review – M.A.S., M.C., D.N., O.A.P., S.M.M.

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