Evaluation of the Effect of Long-term Use of Three Intracanal Medicaments on the Radicular Dentin Microhardness and Fracture Resistance: An in vitro study

Usporedba dugotrajne primjene triju intrakanalnih lijekova na mikrotvrdoću i otpornost na lom korijenskog dentina: in vitro istraživanje

Abstract

Objectives: The aim of this research was to evaluate the effect of long-term use of three intracanal medicaments on the radicular dentin microhardness and fracture resistance. Material and methods: A chemomechanical preparation was done using the Protaper rotary instruments up to F3. The teeth were stored in an incubator at 37°C at 100% humidity and were categorized in three groups by random allocation, namely: Triple Antibiotic Paste (TAP), Calcium hydroxide paste (Apexcal) and Ledermix. Following medicament application, the access openings of all teeth were sealed with 4 mm thickness of cavity. The samples were stored for periods of 1 week, 1 month and 3 months. Two dentin cylinders measuring 5 mm and 3 mm were obtained from each sample. The cervical third was used for fracture resistance and the middle third was used for microhardness evaluation. The microhardness testing was done using a Knoop microhardness tester, and the fracture resistance testing was done using the universal testing machine. Results: Calcium hydroxide showed maximally negative effect on the physical properties of radicular dentin compared to TAP (p = 0.0100 at one month and Ledermix (p = 0.0001 at one month). With an increase in the application time, there was an increased deterioration in the physical properties of radicular dentin. Conclusion: Long-term placement of calcium hydroxide, Triple Antibiotic Paste, and Ledermix (p = 0.0001 at 3 months) significantly affects the microhardness and fracture resistance of radicular dentin.

Introduction

The root canal system is comprised of the main canal and various complexities such as lateral canals, ramifications, loops, isthmuses, deltas (1). In infected root canals, bacteria, which persist deep within the dentin, may be difficult to eradicate and trigger periapical pathosis (2). During a root introdunction

Uvod

Sustav korijenskog kanala sastoji se od glavnog kanala i različitih lateralnih kanala, ramifikacija, petlji, istmusa i delti (1). Zato je u slučaju infekcije teško eliminirati bakterije duboko unutar dentina koje izazivaju periapikalnu patologiju (2). Istaknimo da tijekom instrumentacije korijenskog kana-
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Intracanal Medicaments

Intracanal Medicaments

la gotovo polovina površine samog kanala i njegovih ogranka ostane neobrađena (3).

Adekvatna kemijsko-mehanička preparacija kojom se poštiže trodimenzionalno brvljenje važna je za uspješnu endodontsku terapiju (4). Neuspjeh se najčešće veže za trajnu pri sistematičnih, koji nastavljaju živjeti u dentinu čak i nakon detaljnog čišćenja i širenja kanala (5). Kombinirana upotreba pravilne izolacijske tehnike (suhoga radnog polja), biomehanička preparacija, učinkovite otopine za ispiranje i tehnika rada te dokazano djelotvorno dezinficirajuće sredstvo ključni su za uspješno liječenje korijskog kanala (6).

Lijekovi su u protokolima dugotrajne terapije veoma važni u pripremi korijskog kanala, posebno u slučaju regenerative endodoncije i apektifikacije. Kalcijev hidroksid, trostruk antibiotička pasta (TAP), dvostruka antibiotička pasta (DAP) te Ledermix najčešće se primjenjuju kao intrakanalni lijevici (7).

Kalcijev hidroksid (CH) često je u upotrebi za dezinficirajuću unutrašnju površinu kanala, a koristi se i za poticanje apektogeneze i apektifikacije, u liječenju internih radikularnih resorptivnih defekata, različitih perforacija te u liječenju periapikalne patologije. Upotrebljava se i kao sredstvo za prekrivanje pulpe (8, 9).

Bystrom i suradnici potvrdili su da CH slabo djeluje na bakterije Enterococcus faecalis (10, 11). U mnogim istraživanjima autori su potvrdili da dugotrajna upotreba CH-a nepovoljno utječe na fizička svojstva radikularnog dentina (12). Rosenberg i suradnici opazili su gotovo 50 postotnu redukciju mikrotenzilne snage (MTFS) zuba nakon dugotrajne (7 do 84 dana) upotrebe CH-a (13). Čak je i srednji modul elasticnosti govedeg dentina značajno porastao, čime je postao sklon frakturama (14).

Nedavno su Hoshino i suradnici predstavili noviju formulaciju nazvanu trostruka antibiotička pasta (TAP) koja sa država ciprofloksacin, metronidazol i minociklin (15). Svi navedeni sastojci imaju antimikrobna i antibakterijska svojstva (16).

Trauma i periapikalna patologija nezrelih trajnih zuba ometaju odlaganje minerala prekidanjem dotoka krvi, te tako zaustavljaju razvoj korijska zuba (17). Apektifikacijska tehnika potiče zatvaranje apeksa postavljanjem MTA čepa, ili periodičnih izmjenama intrakanalnih lijekova (18, 19). Druga terapijska mogućnost je regeneracijski endodontski postupak kojim se pokušava zamijeniti oštećene strukture. Prema istraživanju Bystrom, potrebna je potpuna dezinficacija, u većoj mjeri nego u slučaju kliničke endodoncije (21), pa je upotreba održivoga sterilnog mikrokošišta unutar korijskog kanala adekvatnim i neophodnim postupcima prijekao potrebną za nowostvoreno tkivo dobilo dovoljno vremena za svoju organizaciju unutar endodontskog prostora (22).

Dezinficacija korijskog kanala smatra se ključnom zato što infekcija onemogućuje regeneraciju, oporavak i aktivnost matičnih stanica (23, 24). Protokol Američkoga društva za endodonciju (AAE) preporučuje upotrebu TAP-a u regeneracijskim endodontskim postupcima (25), ali i u liječenju ra-
activity (23, 24). The American Association of Endodontics (AAE) protocol advocates the use of TAP in regenerative endodontic procedures (25). Its use is also advocated in the treatment of radicular resorptive defects, root fracture, and also in the non-surgical management of periapical pathosis (26).

The use of TAP is associated with a few limitations; Tooth discoloration is the biggest drawback of this medicament, which arises due to the presence of minocycline which has a negative impact on the esthetics of the patient, especially when used in the anterior teeth (27, 28). TAP, when used for long term, has shown to cause an increased radicular dentin demineralization and an associated reduction in microhardness (22, 29).

Ledermix™ paste is a commercially available intracanal medicament paste that was introduced by Schroeder and Triadan in 1960. It is a polyethylene glycol-based paste containing tetracycline antibiotic, demeclocycline HCl (conc. of 3.2%), and a corticosteroid, triamcinolone acetonide, conc. 1% (30, 31). It is efficient in alleviating pain, in the inhibition of inflammatory root resorption and as an antimicrobial agent (32). In cases of root resorption and large periapical lesions, Ledermix is kept for a prolonged period of time (33, 34). Long term exposure of enamel to Ledermix causes a drastic reduction in the microhardness which is due to the aqueous tetracycline component. Due to lack of literature in this field this study was designed to evaluate the long-term impact of these medicaments on properties such as microhardness and fracture resistance of radicular dentin.

The aim of this work is to compare and evaluate the effect of long-term use of three intracanal medicaments on the radicular dentin microhardness and fracture resistance.

Null hypothesis

There is no difference in the effect of long term placement of calcium hydroxide, Triple Antibiotic Paste and Ledermix on the radicular dentin microhardness and fracture resistance.

Material and methods

Source of data

The study was conducted in Department of Conservative Dentistry and Endodontics, KAHER’s KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research (KLE University) Belagavi.

The microhardness testing was conducted at M.I.T (Manipal Institute of Technology). The fracture resistance testing was performed at K.L.E Engineering College Belagavi. A total of 180 extracted, human permanent single rooted premolar teeth were selected.

Selection criteria

Extracted human permanent single rooted premolar teeth with patent canals and the teeth with apical width corresponding to #20 K-file or less were included. Carious teeth, teeth with apical width more than #20 K-file size, teeth with calcified canals, teeth with fracture/crack or a restoration, teeth with internal/external resorption were excluded.

Nulta hipoteza

Poslije dugotrajne upotrebe nema razlike u učinku kalcijske hidroksida, trostruke antibiotičke paste i Ledermixa kad je riječ o mikrotvrđoći i otpornosti na lom radikularnog dentina.

Materijal i metode

Izvor podataka

Istraživanje je provedeno u Zavodu za restaurativnu dentalnu medicinu i endodonciju KAHER’s KLE VK Institutoz dentalnih znanosti KLE u Belagaviju.

Testiranje mikrotvrđe obavljeno je u Tehnološkom institutu u Manipalu, a otpornost na lom na Inženjerskom fakultetu KLE u Belagaviju. Odabrano je 180 ekstrahiranih jednokorijenskih ljudskih prekrtunjaka.

Kriteriji za odabir

U istraživanje su bili uključeni ekstrahirani jednokorijenski ljudski prekrtunjaci s probodnim kanalima i to oni koji su na apektu imali prohodnost pri uporabi plice br. 20 ili manje. Zubi s karijesom, širim apektima, kalificiranim kanalima, frakturama/pukotinama ili ispunima te zubi s internom/eksternom resorpcijom nisu bili uključeni u istraživanje.
Methodology

180 extracted, human single rooted premolar teeth were selected. Cleaning of visible blood and gross debris was done using an ultrasonic scaler. The extracted teeth were handled according to OSHA (Occupational Safety and Health Administration) guidelines. 0.1% thymol solution was used for the storage of the samples until use.

A round bur with a high-speed handpiece was used to make an access cavity in each tooth specimen. Apical patency was attained using a size 10 K file ((Mani. Inc., Tochigi, Japan). A 15 K-file (Mani. Inc., Tochigi, Japan) was extended 1 mm beyond the apical foramen by visualizing its tip, following which 1 mm was removed from the predetermined working length. Shaping and cleaning was carried out using the Protaper (Dentsply, India) rotary instruments till F3 with endo motor (X-Smart, Dentsply, India). In between each instrumentation change, the canals were irrigated with 2 mL of 3% NaOCl (Vishal Dentocare Pvt. Ltd., Ahmedabad, India), followed by 2 mL of 17% EDTA (DEOR Deo Smear-Off, India) as the final irrigant. The canals were subsequently rinsed with 5 mL of sterile saline using a 27-gauge needle. Following irrigation of teeth, sterile paper points (Dentsply, India) were used to dry the root canals.

The teeth were stored in an incubator at 37°C at 100% humidity. The teeth were stored for periods of 1 week, 1 month and 3 months.

Medicament application

180 specimens were allocated to the following three groups: Group I: Triple Antibiotic Paste (TAP); Group II: Calcium hydroxide paste (Apexcal); Group III: Ledermix

Group I: Triple Antibiotic Paste (TAP)

1st experimental group (n = 60). For the preparation of TAP, 1 mg / ml of each antibiotic powders (USP-grade) in equal quantities of metronidazole, ciprofloxacin and minocycline in a ratio of 3:1 was combined with polyethylene glycol. A sterile lentulo spiral was used for the introduction of the prepared medicament into the root canals with a slow-speed handpiece. Following this, the medicament was compacted to the level of the CEJ using sterile pluggers (Sybron endo).

Group II: Calcium hydroxide paste (Apexcal)

For the second experimental group (n = 60), commercially available Ca (OH) 2 paste (Apexcal) was used. The medicament application to the root canal space was done in a similar manner as described previously.

Group III: Ledermix

For the third experimental group (n = 60), commercially available Ledermix paste was used. The paste was introduced into the root canals as elaborated previously. Following medicament application, 4 mm of cavity (3M ESPE) was used to seal all the prepared specimens. After the coronal seal was achieved, flowable composite (Ivoclar Vivadent) was used to obtain an apical seal.

Preparation of root specimens

A low-speed diamond saw (Agar Scientific) under constant water cooling was used for decoration of the prepared sam-

Metodologija

Odabrano je 180 ekstrahiranih umjetnih jednokorijen- skih premolara. Vidljive i velike naslage očišćene su ultra- zvučnim stružama. Sa zubima se zatim postupalo prema smjernicama OSHA-e (Occupational Safety and Health Administration). Do početka istraživanja čuvani su u 0,1-postotnoj otopini timola.

Pristupni kavitet učinjen je na svakom zubu okruglim svrdlom s pomoću nasadnog instrumenta s velikim brojem okretaja. Prohodnost do apeksa ustanovljena je pilicom br. 10 (Mani Inc., Tochigi, Japan). Plicom br. 15 prošlo se 1 mm preko apikalnog foramina sve do pojave vrha, a taj je mili- metar odbijen od unaprijed određene radne duljine. Obliko- vanje i čišćenje obavljeno je Protaperovim (Dentsply, Indija) rotirajućim instrumentima do F3 s endomotorom (X-Smart, Dentsply, Indija). Između svake upotrebe dviju pila kanali su isprani s 2 mL 3-postotne otopine NaOCl-e (Vishal Den- tocare Pvt. Ltd., Ahmedabad, Indija) i 2 mL 1-postotne oto- pine EDTA-e i 2 mL 1-postotne otopine DEOR Deo Smear-Off, Indija) kao posljed- njoj otopinom. Kanali su na kraju isprani s 5 mL sterilne fiziološke otopine s pomoću igle 27. Nakon irrigacije kanali su potpuno osušeni sterilnim papirnatim štapicima.

Zubi su odloženi u inkubator na 37 °C uz 100-postotnu vlagu i to od tjedan do mjesec dana i tri mjeseca.

Primjena lijekova

Uzorci su podijeljeni u tri skupine: 1 – trostruka antibiotička pasta (TAP); 2 – pasta od kalcijeva hidroksida (Apexcal); 3 – Ledermix

Skupina 1 – trostruka antibiotička pasta (TAP)

U prvoj eksperimentalnoj skupini (n = 60) pomiješan je 1 mg svakoga antibiotičkog praška u jednakim količinama (metronidazol, ciprofloxacin i minociklin) s polietilen glikolom u omjeru 3 : 1. Za unos pripremljenog medicamenta u korijenske kanale koristeno je sterilno lentulo svrdlo s ma- lim brojem okretaja. Nakon toga uložak je sterilnim pluggerima (Sybron endo) komprimiran do caklinsko-cementnog spojista.

Skupina 2 – kalcijev hidroksid (Apexcal)

Za drugu eksperimentalnu skupinu (n = 60) odabrana je komercijalno dostupna pasta Ca(OH), (Apexcal). Postavlja- nje lijeka u korijenski kanal obavljeno je na sličan način kao što je već opisano.

Skupina 3 – Ledermix

Za treću eksperimentalnu skupinu (n = 60) korištena je komercijalno dostupna pasta Ledermix. Unesenje u korijen- jenske kanale kako je već opisano. Nakon toga svaki je uzor- rak zatvoren s 4 mm Cavita (3M ESPE). Nakon koronalnog brtvljenja, apikalno brtvljenje postignuto je tekućim kompo- zitom (Ivoclar Vivadent).

Priprema uzoraka korjenova

Krune su uklonjene dijamantnom pilom s malim brojem okretaja (Agar Scientific) pod konstantnim ispiranjem, 0,5
Intrakanalni lijekovi Amonkar i sur.

Microhardness testing

A Vickers Microhardness Tester (MCS Mechatronic) was used to measure the microhardness of each tooth specimen. Three indentations were created on the polished surface of each specimen with the help of a 50-g load held straight to the polished side for 15 s. The indentations were made 500 μm from the pulp dentin interface. The optical microscope was used to observe indentations created. The mean of the values obtained for the three indentations was representative for each specimen at each depth.

Fracture resistance testing

The Universal testing machine (MCS Mechatronics) was used for assessment on each 5-mm root section. The lower platform was used to place the specimen vertically with the coronal side facing upwards. A cylindrical loading fixture with a radius = 1.9 mm was fixed to the upper crosshead until the root cylinder fractured and was expressed in newtons.

Results

Comparisons of three groups with micro-hardness and fracture resistance at different time points are respectively shown in Figure 1 and Figure 2. Data analysis with Tukey’s multiple posthoc for hardness and fracture resistance is shown in Table 1 and Table 2.

Ispitivanje mikrotvrdoće

Za mjerenje mikrotvrdoće svakog uzorka korišten je uređaj Vickers Microhardness (MCS Mechatronics). Učinjene su tri indentacije na poliranoj površini svakog uzorka s pomoću utega težine 50 grama pritisnutog 15 sekunda na poliranu površinu. Indentacije su napravljene 500 lm od spaja pulpe i dentina. Pregledane su optičkim mikroskopom, a srednja vrijednost svih triju korištena je kao reprezentativna mjera za svaki uzorak na svakoj dubini.

Ispitivanje otpornosti na lom

Za procjenu otpornosti na svakom presjeku korišten je uredaj Universal Testing Machine (MCS Mechatronics). Na donju platformu uzorak je postavljen okomit, s koronalnim dijelom prema gore. Cilindrični vijak promjera 1.9 mm postavljen je na gornju križnu glavu tako da njegov vrh dodiruje uzorak korišten. Primijenjena sila od 0,5 mm u minuti sve dok korijen nije puknuo izražena je u njutnima (N).

Rezultati

Usporedba triju skupina s mikrotvrdoćom i otpornost na lom u različitim vremenskim odsječcima prikazani su na slikama 1 i 2. Analiza podataka Tukeyjevom multiplom posthoc analizom za mikrotvrdoću i otpornost na lom nalazi se u tablicama 1 i 2.
Statistical analysis

The ANOVA test revealed a significant difference in three groups with microhardness and fracture resistance at different time points by one-way ANOVA between all the three groups i.e., Group I (Calcium hydroxide), Group II (Triple Antibiotic Paste) and Group III (Ledermix). The lowest scores for microhardness were shown by Group I (Calcium hydroxide) (49.73±5.25 %), (39.10±3.37 %), (31.09±1.18 %) at 1 week, 1 month and 3 months, respectively. The lowest scores for fracture resistance were shown by Group I (Calcium hydroxide) (737.05±14.92 %), (725.40±15.74 %), (711.35±15.84 %) at 1 week, 1 month and 3 months, respectively. This was significantly different from the other two groups. Group II (TAP) showed the scores for microhardness of (72.72 ± 7.93 %), (61.07 ± 5.42 %), (58.89 ± 6.85 %), and the fracture resistance scores were (752.85 ± 16.76 %), (743.00 ± 16.54 %), (724.55 ± 16.28 %) at 1 week, month and 3 months respectively. Group III (Ledermix) had scores for microhardness of (110.56 ± 28.25 %), (60.00 ± 10.95 %), (57.08 ± 10.64 %), and the fracture resistance scores were (759.75 ± 22.30 %), (752.05 ± 22.18 %), (739.05 ± 22.59 %) at 1 week, 1 month and 3 months respectively. The same values are shown in Figures 1 and 2.

The pairwise comparison of the three experimental groups is described in detail in Table 1 and 2, and a significant difference was found between the Calcium hydroxide (Group I), Triple Antibiotic Paste (Group II), Ledermix (Group III) on the radicular dentin microhardness and fracture resistance.

### Statistical analysis

ANOVA showed a significant difference in the microhardness and fracture resistance of the three groups at different time points. The lowest microhardness scores were observed in Group I (Calcium hydroxide) and the highest in Group III (Ledermix). Similarly, the fracture resistance was highest in Group III (Ledermix) and lowest in Group I (Calcium hydroxide). The pairwise comparisons using Tukey’s post hoc test revealed significant differences between the groups at different time points.

#### Table 1

| Times     | Groups vs          | Groups    | Mean Difference | Std. Error | p-value |
|-----------|--------------------|-----------|-----------------|------------|---------|
| 1 week    | Calcium hydroxide vs • Tap | Tap       | -22.99          | 5.44       | 0.0001** |
|           | Calcium hydroxide vs Ledermix | Ledermix  | -60.83          | 5.44       | 0.0001** |
|           | Tap vs Ledermix    | Tap       | -37.84          | 5.44       | 0.0001** |

#### Table 2

| Times     | Groups vs          | Groups    | Mean Difference | Std. Error | p-value |
|-----------|--------------------|-----------|-----------------|------------|---------|
| 1 week    | Calcium hydroxide vs Tap | Tap       | -15.80          | 5.78       | 0.0220* |
|           | Calcium hydroxide vs Ledermix | Ledermix  | -22.70          | 5.78       | 0.0010** |
|           | Tap vs Ledermix    | Tap       | -6.90           | 5.78       | 0.4610  |

The pairwise comparison of the three groups with microhardness at different time points by Tukey’s multiple posthoc procedures

| Times     | Groups vs          | Groups    | Mean Difference | Std. Error | p-value |
|-----------|--------------------|-----------|-----------------|------------|---------|
| 1 week    | Calcium hydroxide vs Tap | Tap       | -17.60          | 5.81       | 0.0100* |
|           | Calcium hydroxide vs Ledermix | Ledermix  | -26.65          | 5.81       | 0.0010** |
|           | Tap vs Ledermix    | Tap       | -9.05           | 5.81       | 0.2720  |

| Times     | Groups vs          | Groups    | Mean Difference | Std. Error | p-value |
|-----------|--------------------|-----------|-----------------|------------|---------|
| 1 week    | Calcium hydroxide vs Tap | Tap       | -13.20          | 5.85       | 0.0700  |
|           | Calcium hydroxide vs Ledermix | Ledermix  | -27.70          | 5.85       | 0.0010** |
|           | Tap vs Ledermix    | Tap       | -14.50          | 5.85       | 0.0420* |

*p<0.05, **p<0.01
Discussion

Due to the complexity of the root canal system, biomechanical preparation by itself is incompetent in completely eradicating microorganisms. Recent research has revealed that the bacteria present in instrumented canals prior to completion of treatment can increase in number and reach their pretreatment count in about 2-4 days (35). Amongst the persistent bacteria within the root canal *E. faecalis* is the most resilient microorganism that causes persistent periapical lesions. In these cases, the use of intracanal medicaments is encouraged (36). The application time of intracanal medicaments varies depending upon its clinical use which may range from 1 to 4 weeks for decontamination of the root canal and even extend up to 11 weeks in cases of endodontic regeneration.

It has been affirmed that microhardness assessment gives an indirect substantiation of the mineral loss or gain in mineralized dental tissues as it is dependent upon the amount of calcified matrix per square millimeter (37). Arends and Bosch stated that microhardness testing is the most practical method of indirect quantitative analysis by accessing the amount of demineralization of dental hard tissues. Microhardness test provides an insight about the dentinal interaction with different medicaments (36).

Dentin may differ significantly between teeth and is associated with considerable number of variations. The tubular density of dentin has found to increase from cervical to apical areas of radicular dentin, which results in an inverse correlation amongst radicular dentinal microhardness and radicular tubular density. This may cause alterations in the results due to the variances in adjacent areas of the dentinal tissue (38). Hence, in the present study, the microhardness measurement was performed in the middle-third of the root structure for each sample.

Vickers microhardness testing was chosen since it is less affected by measurement errors. Despite small sized samples and surface conditions the specimens can be evaluated with good accuracy (36). There have been cumulative reports of demineralization, associated surface weakening and deterioration in the mechanical properties of radicular dentin which eventually led to microcracks and development of vertical root fracture following long term placement of intracanal medicaments (39). The assessment of the mechanical properties of radicular dentin can be done by fracture resistance studies as it relates to the amount of demineralization. Hence, the fracture resistance evaluation was chosen as the second parameter to be tested in this study. In this study, the force was generated at an angle of 0°, which results in the development of a splitting stress over the tooth specimen. The stresses induced would be minimal because of less bending moments (40). In numerous clinical studies the immature permanent teeth treated by endodontic regeneration have shown an increase in the radicular dentinal wall thickness limited mainly to the middle and the apical third rather than the cervical region, thus weakening this region with an increased incidence of fracture; therefore, the cervical third of the root was chosen for fracture resistance test (29, 41).

Rasprava

Zbog kompleksnosti sustava korijenskog kanala, samo biomehanička preparacija nije dovoljna za potpunu eliminaciju mikroorganizama. Istraživanja su pokazala da broj bakterija u instrumentiranim kanalima prije završetka terapije može po- rasti do predterapijske razine za dva do četiri dana (35). *Enterococcus faecalis* ističe se kao najperzistentnija bakterija koja najčešće uzrokuje perzistirajuće perapikalne lezije. U tom se slučaju preporučuju intrakanalni lijekovi (36). Vrijeme primjene takvih uložaka razlikuje se ovisno o kliničkoj upotrijebi, uglavnom u rasponu od jednog do četiri dojam za dekontaminaciju korijenskog kanala, pa čak do 11 tjedana u slučaju endodontskih regeneracija.

Potvrđeno je da procjena mikrotvrdoće daje direktnu informaciju o gubitku ili dobivku minerala u mineraliziranom dentinu, zato što je ono ovisno o količini kalcificiranog matriksa (37). Arends i Bosch ustanovili su da je ispitivanje mikrotvrdoće najpraktičnija indirektna kvantitativna metoda za procjenu količine demineralizacije tvrdih dentalnih tkiva. Ispitivanje mikrotvrdoće daje uvid u dentinsku interakciju s različitim lijekovima (36).

Dentin se prema sastavu može razlikovati od zuba do zuba, a i povezan je s nizom varijacija. Gustoća tubula dentina povećava se od cervikalnoga prema apikalnom dijelu radikularnog dentina, što je u skladu s njenom količinom demineralizacije (38). Stresi izazvani u radikularnom dentinu može se direktno procijeniti (36). Kumulativni opisi demineralizacije, oštećenja površine i gubitak mehaničkih svojstava radikularnog dentina s vremenom mogu prouzročiti mikropukotine i okomite frakture korijena nakon dugotrajne primjene intrakanalnih lijekova (39). Procjena mehaničkih svojstava radikularnog dentina može se dobiti i istraživanjem odpornosti na lom sastavljeno u srednjem dijelu korijena svakog uzorka.

Odarabano je mjerenje mikrotvrdoće prema Vickersu zato što na njega manje utječe pogreške pri mjerenju te se unatoč sitnim uzorcima i stanju površine uzorke može dobiti dobro precizno procijeniti (36). Katkoli uvijek je kliničko povijest uvijek postoji. Sufinčki miotrofni i apikalni dijelovi stresi se smanjuju s povećanjem površine zona nadmrazak u uložimu. middit u kliničkom usprkos i primjerima demineralizacija (36). Stoga je kompanija protopera u gubitku u cementu na unaprajujući stresi, a i povezan je s različitim lijekovima (36).

Budući da nikal-titanijevi instrumenti iz seta Protaper pokrivaju cijeli raspon liječenja s manjim brojem instrumenta, što uključuje veću fleksibilnost, nemadnašnu učinkovitost i povećanu sigurnost, oni se u preparaciji korijenskih kanala koriste češće nego čelični (54). To je bili standardizirani, svaki je korijenski kanal prepiran Protaperom veličine F3, što odgovara veličini iglice br. 30.
As NiTi Protaper instruments are intended to cover the entire range of treatment with fewer files which integrate greater flexibility, unparalleled efficacy and enhanced safety. They have been used over stainless steel instruments in order to prepare the root canals. For the standardization of the root canal, each root was prepared up to Protaper size F3, which corresponds to ISO #no. 30.

Manual agitation was done carefully in an attempt not to abrade the radicular dentin since it has been advocated that corresponds to ISO #no. 30. Each root was prepared up to Protaper size F3, which allows for the root canals. For the standardization of the root canal, each root was prepared up to Protaper size F3, which corresponds to ISO #no. 30. The results of the present study totally are consistent with the stem cells of dental pulp and apical papilla. Therefore, decreased concentration in a range of 0.1 mg/mL to 2 mg/mL has been recommended.

The lowest scores for microhardness and fracture resistance were shown by Group I Calcium hydroxide at 1 week, 1 month and 3 months, respectively. The present result could be explained by the alkaline pH of calcium hydroxide and its low molecular weight which denatures the collagenous matrix. The results of the present study are consistent with a recently performed study by Yassen et al. which showed that Ca(OH)2 produced substantial collagen degradation of superficially present root canal dentine after a short duration of 1 week (45). It is well understood that the collagen constituent is accountable for the toughness of the mineralized hard tissues, hence any defect produced compromises the mechanical properties making it more susceptible to fracture, which has been seen in this study after 3 months. Long term application of Ca(OH)2 also enhances crack propagation (44).

Increased susceptibility to fracture was seen in this study’s 3-month fracture resistance data. Group II (Triple Antibiotic Paste) showed the scores for microhardness of (72.72±7.93 %), (61.07±5.42 %), (58.89±6.85 %), and the fracture resistance scores were (752.85±16.76 %), (743.00±16.54 %), (724.5±16.28 %) at 1 week, 1 month and 3 months respectively. This may be credited to various acids commonly mixed with antibiotics to preserve their chemical steadiness and to regulate their toxicity. The results of the present study totally reject the null hypothesis.

Minocycline causes chelation of calcium, which in turn contributes to demineralization (44, 45). Studies show that increased concentration of TAP is directly proportional to the surface roughness (46). This also causes reduction in the organic content and loss of dentin, which decreases the wettability of dentin surfaces (47). The results obtained in the present research are in line with studies conducted by Yassen et al. (2013) and Kinney et al. (2003) that demonstrated that 1 g/mL TAP caused severe reduction in microhardness and Manual agitation proved to be nежно како се не би до-
godila abrazija radikularnog dentina, zato что je poznato да zadržavanje intrakanalnog lijeja utječe на zaostatni sloj tijekom instrumentacije (43). Hoshino i suradnici preporučili su korištenje TAP-a (3Mix) u omjeru 1 : 1 : 1 (ciprofloksacin 200 mg, metronidazol 500 mg, minociklin 100 mg). Ciprofloksacin i minociklin imaju minimalnu inhibitornu koncentraciju za E. faecalis od 5 do 20 µg, a metronidazol nema nikakvo inhibitorno djelovanje na tu bakteriju (57). No pokazalo se da kombinacija (100 µg svakog antibiotika/mL) inhibira rast svih vrsta mikroorganizama (58). U regenerativnoj endodonciji povećana koncentracija TAP-a potiče citotoksicne učinke na matičnim stanicama pulpe i apikalne papile. Za to se preporučuje smanjena koncentracija na rasponu od 0,1 mg/mL do 0,2 mg/mL.

Najniže vrijednosti mikrotvrdoće i otpornosti na lom zabijelene su u skupini 1 (kalcijev hidroksid) nakon jednoga tjedna, mjesec dana i tri mjeseca. Ti rezultati možda su prouzročeni alkalnim pH kalcevih hidroksida i njegovom niskom molekularnom masom koja denaturira kolageni matriks (44). Rezultati našeg istraživanja u skladu su s nedavno objavljenim radom Yassen i suradnika u kojem se ističe da Ca(OH)2 izaziva značajnu degradaciju kolagenov površinskog dentina u korijenskom kanalu nakon samo tjedana dana (45). Jasno je da je kolagen odgovoran za čvrstoću mineraliziranog tkiva pa i svaki njegov defekt kompromitira mehanička svojstva tki-vaja koje postaje osjetljivo na frakturu, baš kao što smo i mi potvrdili nakon tri mjeseca. Dugotrajna primjena kalcevih hidroksida potiče širenje pukotina (44). Povećana osjetljivost na lom uočena je u našem istraživanju na uzorcima nakon tri mjeseca. U skupini 2 (TAP) zabilježena je vrijednosti za mikrotvrdoću 72,72 ± 7,93 %, 61,07 ± 5,42 % i 58,89 ± 6,85 %, a za otpornost na lom 752,85 ± 16,76 %, 743,00 ± 16,54 % i 724,5 ± 16,28 % nakon tjedan dana, mjesec dana i tri mjeseca. To se može pripisati različitim kiselinama koje se daju antibioticima da bi se sačuvala njihova kemijska aktivnost te kontrolirao toxicitet. Rezultati dobiveni u ovom istraživanju u cijelosti odbacuju nultu hipotezu.

Minociklin uzrokuje kelaciju kalcija, što pogoduje demineralizaciji (44, 45). Istraživanja pokazuju da je povećana koncentracija TAP-a izravno proporcionalna hrapavosti površine (46). To također smanjuje anorganski sadržaj i gubitak dentina, što smanjuje mogućnost njegova vlaženja (47). Rezultati našeg istraživanja u skladu su s onima koje su objavili Yassen i suradnici koji su naveli da 1 g/mL TAP-a značajno reducira mikrotvrdoću i otpornost na lom radikularnog dentina nakon tjedan i mjesec dana. TAP po-
većava udo organskog sadržaja (29, 48). Razlog za promjene u kemijskim svojstvima radikularnog dentina nakon upotrebe TAP-a jest kiselost (pH < 2,9) pa zbog toga nastaje površinska demineralizacija (29). TAP manje negativno djeluje na kemijsku strukturu radikularnog dentina od CH-a, što smo i mi potvrdili u ovom istraživanju. Vrijeme je važan čimbenik, jer je dulja izloženost izravno proporcionalna s demineralizacijom (26). Rezultati koje su objavili Madhusudhana i suradnici u skladu su s našim istraživanjem (40).

Na uzorcima izloženima Lederminu zabilježena je manja redukcija mikrotvrdoće i otpornosti na lom radikularnog de-
fracture resistance of the radicular dentin at time intervals of 1 week and 1 month. TAP increases the organic content (29, 48). The reason attributed to the change in the chemical properties of radicular dentin following the use of TAP is due to its acidic ability (pH = 2.9) and associated surface demineralization (29). TAP has a less negative effect on the chemical structure of radicular dentin compared to CH which was also found in the present study. Time period being an important factor, the increased exposure time is directly proportional to demineralization (26). The results obtained by Madhusudhana K et al. are consistent with the results of the present study (40).

The samples exposed to Ledermix intracanal medicament showed less reduction in the radicular dentin microhardness and fracture resistance. This could be attributed to its lower pH (8.76) compared to the Ca (OH)₂, intracanal medicament (65). It has been proposed that a higher alkaline pH may cause denaturation of the organic matrix or result in the collapse of the dentine inorganic matrix. Studies have found that the denaturing of organic matrix is promoted by high pH (49). There is encapsulation of the organic matrix by the inorganic hydroxyapatite, and the penetration of the intracanal medicament requires greater time (e.g., 4 weeks in some studies), thus making the dentinal structure more brittle with an increased susceptibility to fracture.

Further in vivo studies are needed in order to apply the results obtained from the present study in clinical setting. There is insufficient evidence to current protocols followed in regenerative endodontics. This has been proposed based upon research and previous case reports. The area of regenerative endodontics is still in its budding stage where we need evidence-based data to determine the best treatment time, the best medicament(s), and the best concentration without compromising the physical properties of the tooth.

Conclusions

Within the parameters of this in vitro study, the null hypothesis has been rejected and the following conclusions can be drawn: The results of the present study suggest avoiding long term use of intracanal medicaments which are generally used in regenerative endodontics to obtain a sterile environment to promote growth. The three-month application of Ca (OH)₂, TAP and Ledermix intracanal medicaments significantly decreased the fracture resistance and microhardness of radicular dentin compared to the 1-week application. A prolonged exposure of intracanal medicaments has adverse effects on chemical and mechanical properties of root canal dentin.

Limitations: Further research is needed in order to optimize the time of application in various long term endodontic procedures and to investigate the demineralization effect of TAP and Ledermix.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.
Conflicts of Interest

The authors declare no conflict of interest.

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