A COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT ENDODONTIC IRRIGATING SOLUTIONS ON MICROHARDNESS OF ROOT CANAL DENTIN : AN IN-VITRO STUDY

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement

for the degree of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. CHRIS CHERIAN GEOGI

Under the guidance of

DR. SANDEEP DUBEY

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

Batch: 2019 - 22 **Enrollment No: 11903222217**

A COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT ENDODONTIC IRRIGATING SOLUTIONS ON MICROHARDNESS OF ROOT CANAL DENTIN : AN IN-VITRO STUDY

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfillment of the requirement for the degree

of

MASTER OF DENTAL SURGERY

In the subject of

CONSERVATIVE DENTISTRY & ENDODONTICS

Submitted by

DR. CHRIS CHERIAN GEOGI

Under the guidance of

DR. SANDEEP DUBEY

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

Batch: 2019 - 22 **Enrollment No: 11903222217**

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCE, LUCKNOW.

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"A comparative evaluation of the effect of different endodontic irrigating solutions on microhardness of root canal dentin : an in-vitro study"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. Sandeep Dubey** , Reader, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BabuBanarasi Das University, Lucknow, Uttar Pradesh.

Date: 30-03-2022

Place: BBDCODS

Signature of the Candidate

Dr. Chris Cherian Geogi

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCE, LUCKNOW.

CERTIFICATE BY THE GUIDE/CO-GUIDE

This is to certify that the dissertation entitled **" A comparative evaluation of the effect of different endodontic irrigating solutions on microhardness of root canal dentin : an in-vitro study"** is a bonafide work done by **Dr Chris Cherian Geogi**, under our direct supervision & guidance in partial fulfillment of the requirement for the degree of **Master of Dental Surgery (M.D.S.)** in the speciality of Conservative Dentistry and Endodontics.

Sandeep Dubey

GUIDE

Dr. Sandeep Dubey

Reader

Department of Conservative Dentistry and Endodontics,

Babu Banarasi Das College of Dental Sciences, Lucknow.

R. Singh

CO-GUIDE

Dr. Palak Singh

Sr. Lecturer

Department of Conservative Dentistry and Endodontics,

Babu Banarasi Das College of Dental Sciences, Lucknow.

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCE, LUCKNOW.

ENDORSEMENT BY THE HOD

This is to certify that this dissertation entitled **"A comparative evaluation of the effect of different endodontic irrigating solutions on microhardness of root canal dentin : an in-vitro study"** is a bonafide work done by **Dr. Chris Cherian Geogi**, under the direct supervision & guidance of **Dr.Sandeep Dubey**, Reader, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BabuBanarasi Das University,Lucknow, Uttar Pradesh.

Dr. B. Rajkumar
Professor & Head
Department of Conservative Dentistry and Endodontics,
Babu Banarasi Das College of Dental Sciences, Lucknow.

PRINCIPAL
Babu Banarasi Das College of Dental Sciences
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226028

DEPARTMENT OF CONSERVATIVE DENTISTRY & ENDODONTICS

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCE, LUCKNOW.

ENDORSEMENT BY THE HEAD OF THE INSTITUTE

This is to certify that this dissertation entitled **"A comparative evaluation of the effect of different endodontic irrigating solutions on microhardness of root canal dentin : an in-vitro study"** is a bonafide work done by **Dr.Chris Cherian Geogi**, under the direct supervision & guidance of **Dr.Sandeep Dubey,** Reader, Department of Conservative Dentistry & Endodontics, Babu Banarasi Das College of Dental Sciences, BabuBanarasi Das University,Lucknow, Uttar Pradesh.

Dr. B. Rajkumar

Principal

Babu Banarasi Das College of Dental Sciences,

Babu Banarasi Das University, Lucknow.

COPYRIGHT

I hereby declare that **BabuBanarasi Das University** shall have the right to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

Date: 30-03-2022

Place: BBDCODS

Signature of the Candidate

Dr. Chris Cherian Geogi

ACKNOWLEDGEMENT

"But if any of you lacks wisdom, he should ask God who gives to all generously and ungrudgingly, and he will be given it. " (James 1:5)

I thank ***God*** *almighty, who by HIS immense mercy, love and guidance enabled me to carry out this work, which I could complete only because of HIS grace.*

It is a profound sense of gratitude that I express my thankfulness to my mentor and guide, ***Dr.Sandeep Dubey, MDS, Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow,*** *who has been a constant source of inspiration and encouragement to me . Throughout this work he patiently beared with me, understood me and accordingly guided me . He selflessly dedicated his time and efforts to nurture this work and give it wings, and I am a witness to this right from the start. The present work bears at every stage the interest of his wise, logical suggestions and meticulous attention to details, which has helped me in bringing this dissertation to its ultimate goal.*

With deep sense of gratitude, I thank and acknowledge my Co-Guide ***Dr. Palak Singh, Assistant Professor, Babu Banarasi Das College of Dental Sciences, Lucknow****. She was one of the most approachable teachers, giving thoughtful suggestions and corrections through her smiling face. She has selflessly set aside her time and energy to bring this work to its completion. I thankfully acknowledge her efforts in imparting quality and fineness to the study.*

I am deeply indebted to respected ***Dean and Professor and Head of the Department, Dr. B. Rajkumar, Babu Banarasi Das College of Dental Sciences, Lucknow****, for his constant support, caring attitude, fatherly advice and interest that has helped me to carry out this work.*

I would like to extend my sincere thanks to ***Dr. Lalit C. Boruah, MDS*** *who had selflessly guided me in the initial days of my post graduate days, who also had been an inspiration and role model to follow.*

I would like to extend my sincere thanks to ***Dr. Vishesh Gupta, MDS, Babu Banarasi Das College of Dental Sciences, Lucknow*** *for all the much needed constructive criticism, along with the ingenious suggestions to overcome them.*

I would like to express my gratitude to ***Dr.Akanksha Bhatt, Ph.D., MDS, Reader, Babu Banarasi Das College of Dental Sciences, Lucknow*** *for extending all cooperation everlasting guidance, motherly care and advice when need arose, for being there when I needed her help.*

I would like to extend my sincere thanks to ***Dr. Tanu Tiwari, MDS, Babu Banarasi Das College of Dental Sciences, Lucknow*** *for all the much needed constructive criticism, along with the ingenious suggestions to overcome them.*

I am truly grateful to ***Dr. Jaya Singh, Dr. Pragya Paliwal, Dr. Ananya Sharma, Dr. Tarun Saxena MDS, and Dr. Rita Gupta, Senior Lecturer, Babu Banarasi Das College of Dental Sciences, Lucknow*** *for their help & support in the completion of my dissertation.*

With deep sense of gratitude, I acknowledge my revered teacher ***Dr. Amit Nigam, Senior lecturer, Babu Banarasi Das College of Dental Sciences, Lucknow*** *for his thoughtful suggestions and efforts in imparting quality, fineness and quality to the study.*

Words cannot describe my emotions for my beloved Parents ***– Dr. Geogi Cherian*** *&* ***Dr. Anitha Jose*** *and my loving brother* ***Jess Joseph Geogi,*** *who always stood by me in times of joy and distress and have given me the strength to face the world.*

I would like to thank my colleagues ***Dr. Aarushi Shekhar, Dr. Ananya Jain, Dr. Divya, Dr. Prachi Mishra, Dr. Rahul Sharma*** *for their valuable suggestions and support whenever I needed.*

I wish my sincere thanks to my seniors, ***Dr. Sankalp Nigam, Dr. Vandana Shukla, Dr. Ruchi Gupta, Dr. Karishma Rao, Dr. Dibyajit Sur & Dr. Anamika Kumar*** *who have been a great source of inspiration and encouragement to me.*

I wish my sincere thanks to my super seniors, ***Dr. Shruthi Mary Sunil, Dr. Swikriti Agarwal, Dr. Pragyan Paliwal, Dr. Garima Singh, Dr. Gitanjali Singh & Dr. Apurva Sharma*** *for all the guidance, support.*

Special thanks to my juniors, ***Dr. Richu Raju, Dr. Atul Krishnan, Dr. Ricku Mathew Reji, Dr. Rimjhim Singh, Dr. Aishwarya Sudha and Dr. Lakshmi Pandey*** *who have been a constant support throughout.*

I would like to acknowledge the support of the non-teaching staff of our department- ***Mr. Parshuram and Mr. Vaibhav.*** *I would also like to acknowledge college management staff-* ***Mrs. Priyanka, Mrs. Succhi, Mr. Santosh, Mr. Girish & Mr. Saurabh*** *for their support.*

I beg forgiveness from those, whose names I have inadvertently missed but they should find solace that their knowledge has been extended to others by way of this thesis.

Once again I thank GOD almighty, whom I trust more than anything else in existence

TO GOD ALONE BE THE GLORY

Dr. Chris Cherian Geogi

ABSTRACT

Microorganisms and their products are the main etiological factors for pulpal and periapical diseases, playing a significant role in the induction and progression of the disease. Hence, chemical auxiliary substances along with mechanical instrumentation of the root canals are used to promote bacterial elimination during endodontic treatment. The purpose of this study is to evaluate the effects of sodium hypochlorite, chlorhexidine gluconate, calcium hypochlorite, and chitosan nanoparticle solution on microhardness of root canal dentin. A total of 95 maxillary central incisor teeth were chosen for this study. The teeth were decoronated and then subjected to root canal preparation using Hyflex CM files till #25 rotary file. The samples were then longitudinally sectioned. The sections were then mounted on to cyanoacrylate resin blocks. The acrylic block sets were sorted into five groups, depending on the irrigating solution being used – Group A : Normal saline(Control), Group B – 3% sodium hypochlorite, Group C – 2% Chlorhexidine digluconate, Group d – 5% Calcium hypochlorite and Group D – 0.2% chitosan nanoparticle solution. Samples in Group A,B,C,D and E were irrigated with their respective irrigating solutions. A standardized volume of 1ml of the respective irrigant was delivered directly on the root canal dentin using a 27 gauge needle. This was repeated by delivering another 1ml of the irrigant after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was again delivered. So after 15 minutes a total of 3ml of the irrigant was delivered. The specimens were then blotted dry to be submitted for dentin microhardness evaluation.. The dentin microhardness was measured with the Vicker's microhardness tester. In each sample, three indentations were made along lines parallel to the edge of the root canal lumen. At each measurement, 1 indentation was made using a 50-g load perpendicular to the indentation surface with a dwell time of 10 seconds. The hardness values for each specimen was obtained as the average of the results for the three indentations. The results were then statistically analysed using one-way ANOVA followed by post hoc Tukey test. The group A– Saline group had the highest microhardness (HV)of 58.43± 2.61 followed by group C- 0.2% Chlorhexidine group(47.39± 1.93), group B- Sodium Hypochlorite group (47.07± 1.04) and group E- Chitosan group (42.62± 1.76). The group D - 5% Calcium Hypochlorite group had the lowest microhardness (HV) of 42.43±1.62. The present in-vitro study indicates that out of all the tested irrigants, calcium hypochlorite was the most effective in reducing root dentin microhardness. However, further in vivo studies are required to test their effectiveness in the clinical situation.

INTRODUCTION

Endodontic therapy is essentially a debridement procedure that requires the removal of the irritants of the root canal and periapical tissue if success is to be gained. The debridement may be carried out in various ways as the case demands and may include instrumentation of the canal, placement of irrigant and medicament. The main goal of instrumentation is to facilitate effective irrigation, disinfection, and filling. [1]

Microorganisms and their products are the main etiological factors for pulpal and periapical diseases, playing a significant role in the induction and progression of the disease. Hence, chemical auxiliary substances along with mechanical instrumentation of the root canals are used to promote bacterial elimination during endodontic treatment. [2]

In root canal treatment, cleaning involves the removal of all contents of the root canal system before and during shaping. Successful cleaning entails the use of instruments to physically remove substances, irrigating systems to flush loosened materials away, and chemicals to dissolve contents from inaccessible regions. Irrigation is presently the best method for the removal of tissue remnants and dentin debris during instrumentation. The simple act of irrigation flushes away loose, necrotic, contaminated materials before they are inadvertently pushed deeper into the canal and apical tissues. Irrigation solutions also provide gross debridement, lubrication, and destruction of microbes and dissolution of tissues. [3]

An ideal irrigant should have these requisite functions: Lubrication, debridement, antimicrobial effect, and dissolution of organic and inorganic material. Unfortunately, there is no single irrigant to date that fulfills all these ideal requisites. [4]

Irrigant solutions used in endodontic therapy might yield changes in the chemical and physical properties of dentin. [5]

Studies on the modes of action and efficacy of various chemical irrigating solutions have shown their direct effects on both organic and inorganic components of root canal dentine. In turn, the mechanical, chemical and physical properties of dentine structure changes. [6]

Irrigation solutions cause alterations in the chemical composition of dentin. The change of Calcium – Phosphorous ratio affects the original proportion of organic to inorganic components, which in turn changes the microhardness, solubility, permeability and surface roughness of dentin. These effects depend on the application time, the concentration of the irrigants and the irrigation protocol used throughout the endodontic treatment. (7)

Microhardness is defined as the resistance to local deformation and is based on the induced permanent surface deformation that remains after removal of load. Any change in the microhardness of the root dentin may adversely affect sealing ability and adhesion of dental material such as resin cements and root canal sealers to dentin, as well as inhibit resistance to bacterial ingress and permitting coronal leakage. (1) (8)

Microhardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth. This method is easy, quick, and requires only a tiny area of specimen surface for testing. Using this technique, the specimen surfaces are impressed with a diamond indenter (a Knoop or a Vickers) at a certain load for a certain period of time. After load removal, diagonals of the indentation are measured with an optical microscope. The hardness number is defined by the ratio between the indentation load and the area of the residual impression, which depends on the indenter shape. (1)

These alterations in the chemical structure caused by the action of endodontic irrigants may in turn affect the mechanical properties of dentin. It consequently leads to the exposure of collagen and eventually causes decrease in dentin micro-hardness. Pashley D et al., suggested that an inverse relation exists between the dentin micro-hardness and density of the dentinal tubules. Reduced micro-hardness may lead to reduction in modulus of elasticity and flexural strength of dentin. (9) Hence the determination of micro-hardness provides an arbitrary assessment of the change in any mineral content of dental hard tissues.

Microhardness is considered as an indirect evidence of mineral changes in root canal dentin. Such changes could affect the adhesive properties of the dentin surface and also affect its interactions with obturation and coronal restorative materials. (4)

The most commonly used endodontic irrigant is 0.5% to 6.25% sodium hypochlorite solution (NaOCl). It is an antibacterial agent capable of dissolving vital and necrotic organic tissue, as well as the organic component of the smear layer. Sodium hypochlorite fragments long peptide chains and chlorinates protein terminal groups, resulting in N-chloramines that are further broken down into other species. The degradation of the organic components by NaOCl solutions can alter adversely dentinal biomechanics by significantly decreasing its elastic modulus and flexural strength. Despite its advantages, NaOCl is insufficient for achieving thorough cleaning and disinfection of the root canal when used alone. Thus, successive rinses with chelating agents and NaOCl solutions have been advocated as an irrigation regimen for efficient smear layer removal. [(7)]

At 1% concentration, NaOCl has shown to be efficient in dissolving organic matter and promote disinfection. Conversely, higher concentrations, such as 5.25%, may cause change in the dentin mechanical properties, as microhardness, because of the proteolytic action of NaOCl solution in the dentin collagen matrix. [(10)]

Cytotoxic activity is a well-known shortcoming of NaOCl that may cause acute injuring effects, if it reaches the periapical area. NaOCl extrusion during root canal therapy is commonly referred to as “hypochlorite accident;” it causes acute immediate symptoms and potentially serious sequelae.

Chlorhexidine (CHX) is a synthetic material comprising two biguanide groups and two symmetric 4-cholorophenyl rings, connected by a hexamethylene chain. Mechanism of action CHX is a hydrophobic and lipophilic molecule that interacts with lipopolysaccharides and phospholipids on the bacterial cell membrane. The beneficial effect of CHX is due to the interaction of its positive charge with the negatively charged phosphate on bacteria cell walls and its ability to alter the osmotic equilibrium of bacteria cells. This can increase cell wall permeability and allow CHX to penetrate the cell. At high concentrations (≥2%), CHX is a bactericide, as it causes precipitation of cytoplasmic contents; at a lower concentration (0.2%) it causes phosphorous and potassium to leak out of cell structures. [(11)]

Dentin medicated with CHX acquires antibacterial substantivity. The absorption of positively charged ions released by CHX prevents bacterial colonization on the dentin surface, and the duration of this effect exceeds the period of medicament application. [(12)]

Although CHX is useful as a final irrigant, its use as a main endodontic irrigant of the canal is not advised due to its inability to dissolve necrotic remnants and to the fact that it is less effective against gram-negative microorganisms than against gram-positive microorganisms.

In endodontics, CHX is used in two concentrations (0.2% and 2%), and its mode of antibacterial activity is related to concentration. CHX is bacteriostatic at a concentration of 0.2% and bactericidal at a concentration of 2%. [11] The principal challenge that prevents the use of CHX as a routine irrigant in endodontics is its lack of tissue solubility during chemomechanical preparation.

In order to overcome all the drawbacks of irrigants discussed above, there is a need to find a new effective and safer irrigant for the root canal treatment.

Calcium hypochlorite ($Ca[OCl]_2$) is a white powder used for industrial sterilization, bleaching, and purifying water treatment. It is relatively stable and has greater available chlorine compared with NaOCl. Its incorporation in water can be more accurate than preparations by dilution of a more concentrated solution, which can be an advantage for clinical use. De Almeida et al. showed that Ca(OCl)2 associated with ultrasonic irrigation is efficient to reduce root canal contamination and can aid in chemomechanical preparation, and it was as effective as NaOCl. [4] [13]

More recently, calcium hypochlorite (Ca[OCl]2) has been investigated in endodontics, presenting an effective antimicrobial action against Enterococcus faecalis, [14] ability to promote organic tissue dissolution [7], chemical stability [10] and biocompatibility. [1] [2]

Clinical studies have revealed persistence of bacteria within the root canal system in spite of cleaning, shaping and application of highly efficient antimicrobial agents. The most probable reason for this, apart from the general emergence of resistant pathogens, might be the complex anatomy of the root canal system that allows localization of bacteria in the inaccessible areas for antimicrobial agents. Moreover, the efficacy of antibacterial agents can be restricted by factors like concentration, time and volume within the root canals. Development of novel antimicrobial delivery systems to improve the pharmacological characteristics of the applied antibacterial agents has been considered as a part of the solution for this problem. Nanoparticles (NPs) as one of the novel strategies have been at the center of attention in the past few decades owing to their innovative and functional properties. Particles with dimensions of 1–1000 nm, (commonly 5–350 nm in diameter) made from any type of biocompatible substance can be defined as NPs. These systems can greatly improve the therapeutic efficacy of pharmaceutical products by producing more favorable drug bioavailability, serum stability and pharmacokinetics. According to the literature, nano-based formulations provide better penetration and allow slow and controlled release of active ingredients at target sites.

In nanotechnology, a decrease in dimensions up to the atomic level leads to a considerable increase in surface area of the agent; therefore, the contact of NPs with the microorganisms and accordingly the effective interaction with the membrane of pathogen increases. In brief, high surface areas of NPs and consequently higher concentrations at target site are the most effective factors in antimicrobial behaviour when compared with their conventional counterparts. (15)

Chitosan is a natural polysaccharide, prepared by the deacetylation of chitin, which is obtained from the shells of crabs and shrimps. This polysaccharide is endowed with properties of biocompatibility, biodegradibility, bioadhesion to human cells. (16) It also presents low cost, in addition to high chelating capacity for different metallic ions. Its mechanism of action is not yet clear, but it is thought that adsorption, ionic exchange and chelation property may be responsible for formation of the complex between substance and metallic ions.

The Chitosan polymer consists of a chain of many dimers of chitin. The dimers of chitin have two nitrogen atoms and two free electrons that are liable for the interaction of ions between the chelating agent and the metal. In the acidic medium, protonation of amino acid results in a complete position change (-NH3+) which is responsible for the attraction of other molecules for adsorption to occur. (17)

AIM & OBJECTIVES

AIM OF THE STUDY:

The aim of this study was to compare and evaluate the effect of Sodium Hypochlorite, Chlorhexidine, Calcium Hypochlorite and Chitosan nanoparticle solution on the microhardness of root canal dentin

OBJECTIVES

1. To evaluate and compare the effect of sodium hypochlorite on microhardness of root canal dentin.
2. To evaluate and compare the effect of chlorhexidine on microhardness of root canal dentin.
3. To evaluate and compare the effect of calcium hypochlorite on microhardness of root canal dentin.
4. To evaluate and compare the effect of Chitosan nanoparticle solution on microhardness of root canal dentin.
5. To evaluate and compare the effect of the tested irrigating solutions amongst each other.

REVIEW OF LITERATURE

1. **Pashley D, Okabe A, Parham P (1985)** [9] evaluated the correlation between dentin microhardness and dentin tubule density in normal human permanent teeth. A new technique was developed which permitted serial determinations of the microhardness and tubule density of the same group of tubules, beginning near to the dentino-enamel junction and progressing to the pulp chamber. The results showed that there is a highly statistically significant inverse correlation between dentin microhardness and tubular density. Tubular density increased as the pulp chamber was approached. This was associated with a decrease in dentin microhardness, presumably due to a decrease in the amount of intertubular detitin and an increase in individual tubular diameter.

2. **Saleh AA, Ettman WM (1999)** [6] evaluated the effect of several endodontic irrigation solutions on the microhardness of root canal dentine. The canal portions in the root segments included in the first group were irrigated with 3% H_2O_2 and 5% NaOCl solutions used alternatively, while 17% EDTA solution was the irrigation used in the second group. After irrigation, dentine microhardness was re-assessed and compared with the control values obtained before the irrigation treatment. The results showed that, irrigation with either H_2O_2/NaOCl or EDTA decreased the microhardness value of root dentine. Irrigation with EDTA gave more reduction of dentine hardness compared to H_2O_2/NaOCl irrigation.

3. **Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. (2001)** [18] tried to test the null hypothesis that sodium hypochlorite irrigation of root canals does not alter the properties of dentine and contribute to the weakening of root-treated teeth. The effect of two concentrations (0.5%, 5.25%) of sodium hypochlorite (NaOCl) and saline on (i) the elastic modulus and flexural strength of machined dentine bars, and (ii) changes in strain of ‘whole’ extracted human teeth were evaluated. One hundred standardized plano-parallel dentine bars (> 11.7 × 0.8 × 0.8 mm) were randomly divided into the three groups, immersed for 2 h in the respective solutions and then subjected to a three-point bend test. Each tooth had its crown and enamel reduced and root canal prepared. These were irrigated sequentially in a series of four separate,30-minute regimes; initial-saline, 0.5% NaOCl, 5.25% NaOCl and final-saline. There was a significant decrease in elastic modulus of the dentine bars immersed in 5.25% NaOCl compared with the saline

group There was also a significant decrease in flexural strength of the dentine bars in the 5.25% NaOCl group compared to both the saline and 0.5% NaOCl groups.The strain data from the nondestructive tooth loading tests revealed significant increases in tensile strain between the initial-saline and the final-saline stages. Significant increases in compressive strains were also found between initial-saline and 5.25% NaOCl; and between 0.5% NaOCl and 5.25% NaOCl stages.The null hypothesis was rejected, 5.25% NaOCl reduced the elastic modulus and flexural strength of dentine. Irrigation of root canals of single, mature rooted premolars with 5.25% NaOCl affected their properties sufficiently to alter their strain characteristics when no enamel was present.

4. Grigoratos D, Knowles J, Ng YL, Gulabivala K (2001) [(19)] evaluated the effect of sodium hypochlorite (NaOCl) solutions (3%, 5%) and saturated calcium hydroxide (Ca(OH)2) solution, individually and consecutively, on the flexural strength and modulus of elasticity of standardized dentine bars. The dentine bars in the five test groups were treated by exposure to the following solutions; group 2 – 3% NaOCl, 2 h; group 3 – 5% NaOCl, 2 h; group 4 – saturated Ca(OH)2 solution, 1 week; group 5 – 3% NaOCl, 2 h and then saturated Ca(OH)2 solution 1 week; group 6 – 5% NaOCl, 2 h and then saturated Ca(OH)2 solution 1 week. The dentine bars were then loaded to failure in a three-point bend test.The was a significant decrease in the modulus of elasticity and flexural strength of the dentine bars treated with 3% and 5% NaOCl. There was no significant difference in the flexural strength and the modulus of elasticity between the 3% and 5% NaOCl groups. Exposure to Ca(OH)2 significantly reduced the flexural strength but had no significant effect on the modulus of elasticity. The groups treated with sodium hypochlorite followed by calcium hydroxide did not have moduli of elasticity and flexural strengths that were significantly different from those treated only with sodium hypochlorite. It was concluded that NaOCl (3 & 5%) reduced the modulus of elasticity and flexural strength of dentine. Saturated Ca(OH)2 reduced the flexural strength of dentine but not the modulus of elasticity. Sequential use of NaOCl and Ca(OH)2 had no additional weakening effect.

5. Doğan H, Çalt S.(2001) [(20)] Effects of combined and single use of EDTA, RC-Prep, and NaOCl on mineral content of root dentin were evaluated in vitro using energy dispersion spectrometric microanalysis. Thirty-six standardized midroot dentin specimens obtained from human anterior teeth were used. Specimens were polished and divided into six experimental groups. The first two groups were treated with EDTA or RC-Prep followed by NaOCl irrigation. Groups 3 to 5 were treated with EDTA, RC-Prep, and NaOCl, respectively. The last group was irrigated with saline solution as a control. Levels of calcium, phosphorus, and magnesium were measured in the root dentin after treatments. The results showed that (i) EDTA combined with NaOCl irrigation as final flush and NaOCl alone changed the calcium/phosphorus ratio of root dentin significantly ($p < 0.05$); and (ii) there was a significant increase in the magnesium level after the use of chelating agent combined with NaOCl ($p < 0.05$). It was concluded that using NaOCl irrigation as final flush altered the effectiveness of chelating agents on root dentin

6. **Slutzky-Goldberg I, Maree M, Liberman R, Heling I. (2004)** [21] evaluated the effect on root dentin microhardness of 2.5% and 6% sodium hypochlorite solutions for various irrigation periods. A decrease in microhardness was found between the control and samples irrigated with 6% NaOCl and 2.5% NaOCl at all irrigation periods.The decrease in microhardness was more marked after irrigation with 6% NaOCl than 2.5% NaOCl.

7. **Ari H, Erdemir A, Belli S. (2004)** [8] evaluated the effect of 0.2% chlorhexidine gluconate on the microhardness and roughness of root canal dentin compared with widely used irrigation solutions - 5.25% NaOCl ,2.5% NaOCl , 3% H2O2, 17% EDTA, and 0.2% chlorhexidine gluconate. The results indicated that all the irrigation solutions except chlorhexidine significantly decreased microhardness of rootcanaldentin($p <0.05$); 3% H2O2 and 0.2% chlorhexidine gluconate had no effect on roughness of the root canal dentin ($p > 0.05$). According to the results of this study,0.2% chlorhexidine gluconate showed to be an appropriate endodontic irrigation solutions because of its harmless effect on the microhardness and roughness of root canal dentin

8. **Oliveira LD,Carvalho CAT, Nunes W, Valera MC, Camargo CHR, Jorge AOC (2007)** [5] evaluated the effects of endodontic irrigants on the microhardness of rootcanal dentin. The specimens were randomly divided into 3

groups , according to the irrigant solution used: group 1, control (saline solution) , group 2, 2% chlorhexidine gluconate solution; and group 3, 1% sodiumhypochlorite (NaOCl). Data obtained were analyzed using analysis of variance and the Tukey test (5%). Specimens irrigated with 2% chlorhexidine (group 2) or 1% NaOCl (group 3) presented lower values of dentin microhardness, with significant difference in relation to the control group ($P < .05$). It could be concluded that chlorhexidine and NaOCl solutions significantly reduced the microhardness of root canal dentin at 500 micrometer and 1000 micrometer from the pulp-dentin interface.

9. Sayin TC, Serper A, Cehreli ZC, Otlu HG(2007) [22] The purpose of this study was to evaluate the effect of single and combined use of ethylenediamine tetra acetic acid (EDTA), ethylene glycol bis [b-aminoethylether] N,N,N',N'-tetraacetic acid (EGTA), EDTA plus Cetavlon (EDTAC), tetracycline-HCl, and NaOCl on the microhardness of root canal dentin.The crowns of 30 single-rooted human teeth were discarded at the cementoenamel junction and the roots were bisected longitudinally to obtain root halves (N = 60). The specimens were embedded in autopolymerizing acrylic resin, leaving the root canal dentin exposed. Dentin surfaces were prepared for microhardness test by grinding and polishing. The reference microhardness values of untreated specimens were recorded using a Vicker's microhardness tester at the apical, midroot, and cervical levels of the root canal. Thereafter, the specimens treated with single (test solution only) or combined (test solution, followed by 2.5% NaOCl) versions of the irrigants for 5 minutes. Posttreatment microhardness values were obtained as with initial ones. All treatment regimens except distilled water significantly decreased the microhardness of the root canal dentin (P < . 05). The single and combined use of EDTA decreased the microhardness of the root canal dentin significantly more than all other treatment regimens (P < .05). Compared with their single-treatment versions, all combined treatment regimens decreased the mean microhardness values significantly (P < .05). A comparison of single and combined treatment regimens revealed significant decreases only for EDTA and EDTA + NaOCl in the coronal region and for EDTAC and EDTAC + NaOCl in the apical and middle regions of the root canal (P < .05).It was concluded thatThe use of EDTA alone or prior to NaOCl resulted in the maximum decrease in dentin microhardness. The softening effect of subsequent NaOCl treatment was both material and region dependent. However, for combined treatment regimens, subsequent use of NaOCl levels the statistical differences between the regional microhardness values obtained after treatment with EGTA, EDTAC, and tetracycline-HCl.

10. Patil CR, Uppin V (2011) [(14)] evaluated the effect of widely used endodontic irrigating solutions on root dentin microhardness and surface roughness. The solutions used were 5% and 2.5% NaOCl solutions, 3% H2O2 , 17% EDTA solution, 0.2% chlorhexidine gluconate, and distilled water. Then, the specimens were subjected to microhardness and roughness testing. The results of this study indicated that all irrigation solutions, except 0.2% chlorhexidine gluconate, decreased the microhardness of root dentin, and 3% H2O2 and 0.2% chlorhexidine gluconate had no effect on surface roughness. Within the limitation of this study, it was concluded that 0.2% chlorhexidine gluconate showed to be an appropriate irrigation solution, because of its harmless effect on the microhardness and surface roughness of root canal dentin.

11. Cruz-Filho AM, Sousa-Neto MD,Savioli RN, Silva RG, Vansan LP, Pecora JD (2011) [(23)] evaluated the effect of different chelating solutions on the microhardness of the most superficial dentin layer from the root canal lumen. Irrigants were distributed in seven groups according to the final irrigation: 15%EDTA, 10%citric acid, 5% malic acid, 5% acetic acid, apple vinegar, 10% sodium citrate, and control (no irrigation). It was concluded that, except for sodium citrate, all tested chelating solutions reduced the microhardness of the most superficial layer of dentin of the root canal lumen. EDTA and citric acid were the most efficient.

12. Silva PV, Guedes DF, Pécora JD, Cruz-Filho AM. (2012) [(24)] evaluate the effects of chitosan at different concentrations on the removal of the smear layer and on dentinstructure after 3 and 5 min of application. The specimens were distributed according to the time and concentration of the final irrigating solution: G1: 0.1% chitosan for 3 min; G2: 0.2% chitosan for 3 min; G3: 0.37% chitosan for 3 min; G4: 0.1% chitosan for 5 min; G5: 0.2% chitosan for 5 min; G6: 0.37% chitosan for 5 min. G1 exhibited removal of the smear layer, but not the smear plugs. G2 showed visible and open tubules with slight erosion of the peritubular dentin. Cleaning in G3 was similar to that in G2, however, the erosive effect was greater. There was expansion of the diameter of the tubules in G4; and in G5 and G6, there was severe erosion with deterioration of dentin surface. In conclusion, 0.2% chitosan for 3 min appeared to be efficient for removing the smear layer, causing little erosion of dentin.

13. Dutta A, Saunders WP(2012) [25] compared the tissue-dissolution properties of 5% and 10% calcium hypochlorite (Ca(OCl)2) with two concentrations (1.36% and 4.65%) of proprietary sodium hypochlorite (NaOCl) on bovine muscle tissue. The available chlorine concentration of each solution was determined using iodometric titration. Available chlorine concentrations of the irrigants ranged from1.36% to 4.65%. All solutions dissolved tissue completely. Chlorax (4.65% NaOCl) (Cerkamed Group, Nisko, Poland) dissolved tissue quicker during the first 35 minutes Within the limitations of this study, it was concluded that Chlorax (4.65%NaOCl) dissolved tissue faster than the Ca(OCl)2 solutions and Tesco thin bleach (1.36% NaOCl) over the first 35 minutes, but there were no significant differences among the solutions thereafter.

14. Pimenta JA, Zaparolli D, Pécora JD, Cruz-Filho AM. (2012) [16] evaluated the effect of solutions of 0.2% chitosan, 15% EDTA and 10% citric acid on the microhardness of root dentin. All solutions reduced the microhardness of root dentin in a way that was statistically similar to each other.The SEM micrographs showed that the three solutions removed smear layer from the middle third of the root canal.0.2% chitosan, 15% EDTA and 10% citric acid showed similar effects in reducing dentin microhardness.

15. Ulusoy Öİ, Görgül G(2013) [26] compared the effects of different irrigants on root dentine microhardness, erosion and smear layer removal.The teeth were divided into six groups according to the final irrigants used: Group 1: 17% ethylenediamine tetra-acetic acid (EDTA) + 2.5% NaOCl, Group 2: 7% maleic acid (MA) + 2.5% sodium hypochloride (NaOCl), Group 3: 1.3% NaOCl + mixture of tetracycline, acid and detergent (MTAD), Group 4: Smear Clear + 2.5% NaOCl, Group 5: 5% NaOCl, Group 6: saline. Maleic acid showed the greatest reduction in dentine microhardness followed by EDTA and MTAD.

EDTA, maleic acid, MTAD and Smear Clear removed smear layer efficiently in the coronal and middle thirds of root canal. However, in the apical region, maleic acid showed more efficient removal of the smear layer than the other irrigants.

16. Garcia et al (2013) (27) evaluated the effect of three different formulations of sodium hypochlorite on the microhardness of root canal dentin in cervical and apical segments. The specimens were divided into three groups according to the sodium hypochlorite formulation used: group 1, 2.5% sodium hypochlorite; group 2, Chlor-XTRA; and group 3, 5.5% sodium hypochlorite gel.. In each group, samples showed reduced dentin microhardness. No differences were observed between the groups, independent of the analyzed segment.All substances showed reduced dentin microhardness. Chlor-XTRA and 5.5% sodium hypochlorite gel promoted a reduction similar to the 2.5% sodium hypochlorite solution.

17. Das A, Kottoor J, Mathew J, Kumar S, George S. (2014) (28) compared the changes in microhardness of root dentin caused by two novel irrigation regimens with conventional irrigation. Samples were divided into four groups according to the irrigation regimen used.Group I: 5% sodium hypochlorite (NaOCl) + 17% ethylenediaminetetraacetic acid (EDTA) + 0.2% chlorhexidine digluconate (CHX) (conventional). Group II: 6% Morinda Citrifolia Juice + 17% EDTA (MCJ). Group III: 5% NaOCl + Q Mix 2 in 1 (QMix). Group IV: Distilled water (control). Irrigation regimens were performed for 5 minutes.Distilled water group showed the least reduction in microhardness when compared with the other groups**.** Within the limitation of this study, it was concluded that NaOCl + Q Mix were least detrimental to root dentin microhardness when compared with MCJ and conventional irrigation regimens.

18. Kandil HE, Labib AH, Alhadainy HA (2014) (29) compared the effect of different irrigants on root dentin microhardness and smear layer removal. There were 5 groups Group 1: 2.5% NaOCl, Group 2: 2.5% sodium hypochloride (NaOCl) followed by 7% malic acid (MA), Group 3: 2.5% NaOCl followed by 17% ethylenediamine tetraacetic acid (EDTA), Group 4: 2.5% NaOCl followed by mixture of tetracycline, acid and detergent (MTAD) and Group 5: saline. Malic acid showed the greatest significant reduction in dentin microhardness followed by EDTA, MTAD, NaOCl and saline (control). EDTA, malic acid and MTAD efficiently removed smear layer, respectively, in the coronal and middle thirds of root canal. However, in the apical region, malic acid showed more efficient removal of the smear layer than the other irrigants. Malic acid was found to be the most efficient final irrigant solution after NaOCl irrigation throughout instrumentation.

19. Aslantas EE, Buzoglu HD, Altundasar E, Serper A. (2014) [30] evaluated the effects of root canal irrigants on the microhardness of root canal dentin in the presence and absence of surface-modifying agents. The samples were divided into 6 groups and treated for 5 minutes with one of the following irrigants: 17% EDTA, REDTA, 2% chlorhexidine gluconate (CHX), 2% CHX with surface modifiers (CHX-Plus), 6% NaOCl, or 6% NaOCl with surface modifiers (Chlor-XTRA). EDTA, REDTA, NaOCl, and Chlor-XTRA significantly decreased the microhardness of root dentin compared with intact controls. It was concluded that the addition of surface modifiers to the irrigants did not affect the microhardness of the samples.

20. Kalluru SR et al (2014) [31] evaluatd the microhardness of human dentin by using four irrigating solutions.Four test groups were formed group 1 with 17% EDTA, group 2 with 17% EDTAC, Group 3 with 3% NaOCl and group 4 with MTAD. Chelating agents EDTA, EDTAC drastically reduced the microhardness of root canal dentin, hence these agents should be used carefully. NaOCl and MTAD not altered the microhardness of root canal dentin significantly. Within the limitations of this study it is concluded that MTAD seems to be an appropriate irrigating solution, because of its harmless effect on the microhardness of root canal dentin.

21. Oliveira JS, RaucciNeto W, Faria NS, Fernandes FS, Miranda CE, Rached-Junior FJ. (2014) [32] Chemical solutions play important roles in endodontic treatment and promote ultrastructural changes in dentin surface. The aim of this study was to quantify root canal roughness at different concentrations of calcium hypochlorite (Ca(OCl)2) and sodium hypochlorite (NaOCl) by confocal laser scanning microscopy (CLSM). Fifty-two human mandibular premolars were sectioned and randomly organized into thirteen groups (n=8):

saline (control); 1%, 2.5% and 5% NaOCl; 1%, 2.5% and 5% Ca(OCl)2; the hypochlorite groups were further divided into with or without EDTA. The chlorine concentrations of the different solutions were measured by iodine titration (%). The superficial roughness (Sa) was quantified by CLSM. Ca(OCl)2 presented substantial decrease in chlorine concentration that differed from the package indication, but without compromising the dentin ultrastructure changes. There were no significant differences in dentin roughness between Ca(OCl)2 or NaOCl at all studied concentrations. The combination with EDTA provided similar roughness values among the solutions ($p>0.05$). The 5% Ca(OCl)2 and NaOCl solutions significantly increased dentin roughness and did not differ from the EDTA association ($p>0.05$). Ca(OCl)2 promoted similar dentin roughness as the NaOCl at the same concentrations and combined with EDTA. It may be concluded that Ca(OCl)2 modified the root canal dentin roughness similarly to NaOCl, at the same concentrations and EDTA combinations used in this study. Ca(OCl)2 and NaOCl, both at 5%, significantly altered dentin roughness, overcoming EDTA association, thus Ca(OCl)2 concentrations ranging from 1% to 2.5% may be suitable solutions for root canal irrigation protocols.

22. Bakr DK, Saleem SS, Amin BK. (2016) [33] evaluated the effects of root canal irrigants on the microhardness of root canal by using three types of irrigant solutions with different concentrations. The irrigants tested were normal saline (control group), 0.2 % chlorhexidin, 2% chlorhexidin, 2.5% sodium hypochlorite, 5.25%, 5% Ethylene dimetha tetra hydrate EDTA and 17% EDTA. After surface treatment, the dentin microhardness of the root samples were recorded at the mid-root level by using a vicker microhardness tester. EDTA, sodium hypochlorite, and 2% chlorhexidin significantly decreased the microhardness of root dentin compared with controls while 0.2% chlorhexidin had no significant effect on the microhardness of root dentin. It was concluded that the irrigant solutions affected the microhardness of the samples except 0.2%.chlorhexidine

23. Saleh HA(2016) [34] evaluated the microhardness of root canal dentin after irrigation with different irrigant solutions for different periods. Samples were divided into five groups; G1 (control) distilled water, G2: 5.25% sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (1 min), G3: 5.25%

sodium hypochlorite (NaOCl) for (10 min) then 17% EDTA for (5 min), G4: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (1 min) and G5: 5.25% sodium hypochlorite(NaOCl) for (20 min) then 17% EDTA for (5 min). The results indicated that all treatment time with 5.25% NaOCl and 17% EDTA decreased dentin microhardness significantly compared to distilled water (control). Treatment with distilled water (control) showed significantly the highest microhardness value, while 5.25% sodium hypochlorite for 20 minute followed by 5 minutes (G5) with 17% EDTA showed significantly the least microhardness value followed by G4, G3 and G2. It was concluded that increasing irrigation time with both 5.25% sodium hypochlorite and 17% EDTA decreased dentin microhardness.

24. Baldasso FE, Roleto L, Silva VD, Morgental RD, Kopper PM. (2017) [(35)] evaluated the effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. The irrigants tested were QMiX, 17% EDTA, 10% citric acid (CA), 1% peracetic acid (PA), 2.5% NaOCl (solution control), and distilled water (negative control).The chelating solutions were used to irrigate the canal followed by 2.5% NaOCl as a final flush. After the irrigation protocols, all specimens were rinsed with 10 mL of distilled water to remove any residue of the chemical solutions. Before and after the final irrigation protocols, dentin microhardness was measured . All protocols significantly reduced dentin microhardness. CA was the irrigant that caused more extensive erosion in dentinal tubules, followed by PA and EDTA. QMiX opened dentinal tubules, but did not cause dentin erosion. Results suggested that QMiX and 17% EDTA reduced dentin microhardness at a greater depth. Additionally, QMiX did not cause dentin erosion.

25. Massoud SF, Moussa SM, Hanafy SA, El Backly RM. (2017) [(36)] evaluated the effect of different irrigation protocols on microhardness of human root canal dentin. The teeth were instrumented using manual stainless steel files and irrigated by 2ml distilled water between each file, then were sectioned by longitudinal splitting of each tooth. Samples were divided into four groups Group I: 10 ml of 2.5% Sodium Hypochlorite, Group II: 10 ml of 17% EDTA followed by 10 ml of 2.5% NaOCL, Group III: 10 ml of 2.5% NaOCL followed by 10 ml of 2% chlorhexidine digluconate (CHX), Group IV: 10 ml of 2.5% NaOCL followed by 10 ml distilled water then were followed by 10ml of 2% CHX. Ten root halves from each group were prepared to measure dentin microhardness at baseline measurement and after treatment to determine the change in microhardness using Vickers tester. Group II showed the highest percentage decrease in microhardness values, followed by group III, then group IV and the lowest was group I. It was concluded that chlorhecidine digluconate was the best final irrigant if there is intermediate flush for prevention of its precipitation with NaOCL.

26. Saha SG et al(2017) (17) evaluated the effect of various endodontic irrigants on the micro-hardness of the root canal dentin. The samples were divided into four groups i.e., 3% Sodium Hypochlorite (3% NaOCl), 17% Ethylene Dioxide Tetra Acetic Acid (17% EDTA), 0.2% Chitosan and 6% Morindacitrifolia Juice (MCJ) . The results of the present study indicated that 17% EDTA and 0.2% Chitosan, significantly decreased the micro-hardness of root dentin whereas 6% MCJ and 3% NaOCl had no significant effect on the microhardness.

27. Abbas FS,Abdulredah NJ,Hassan AS.(2018) (1) evaluated dentin microhardness of root canal dentin after irrigating with different solutions - 2.5% sodium hypochlorite, EDTA 17%, Citric acid 40%, and distilled water as control. The results showed that EDTA group showed maxium reduction in dentin microhardness after final irrigation protocol, followed by the sodium hypochlorite group and the least with citic acid .

28. Taffarel et al (2018) (2) evaluated the effect of irrigation with different root canal irrigants on the microhardness of root dentin.The agents used were 2% chlorhexidine solution, 6% sodiumhypochlorite solution, 6% calcium hypochlorite, Q Mix, 6.5% grape seed extract solution with distilled water as control. All the tested irrigantb solutions maintained the same microhardness level of the root dentin when compared to the control group, with no statistically significant difference between them.($p < 0.05$)

29. Duvvi SAB, Adarsha MS, Usha HL, Ashwini P, Murthy CS, Shivekshith AK.(2018) (4) assessed the effect of different concentrations of sodium hypochlorite and calcium hypochlorite on microhardness of root canal dentin. . The study aimed to assess the effect of different concentrations of sodium hypochlorite and calcium hypochlorite on micro hardness of root canal dentin. . All irrigating solutions showed reduction in microhardness of root canal dentin except saline. 5% NaOCl and 10% CaOCl2 showed maximum reduction in microhardness. 2.5% NaOCl shown least reduction in microhardness followed by 5% CaOCl2. 2.5% NaOCl and 5% CaOCl2 shown less reduction in microhardness of root canal dentin when compared to 5% NaOCl and 10% CaOCl2.

30. Quteifani M, Madarati AA, Layous K, Tayyan MA (2019) [(37)] compared the effects of different irrigation protocols, with/without laser activation, on the radicular dentine's micro-hardness. Eighty-two human extracted premolars were decoronated and divided into 7 groups. Roots were longitudinally split into two halves. The micro-hardness was measured for one half before and after irrigation protocols. The groups were; G1: MTAD without laser-activation, G2: MTAD with laser-activation, G3: sodium-hypochlorite (SH) with laser-activation, G4: SH then EDTA with laser-activation, G5: SH then MTAD with laser-activation, G6: SH without laser-activation. G7: distilled water (control). In the two-irrigants groups G4 and G5), samples were irrigated first with SH then with MTAD or EDTA irrigants, which were activated by the laser. The micro-hardness reduction of SH without laser-activation group was the greatest. The single irrigant or laser-activation irrigation protocols caused significantly less micro-hardness reduction compared to the two-irrigants or no laser-activation protocols. The mean micro-hardness reduction of SH and MTAD groups (both with laser-activation) were significantly lower than other groups, but not from that of the control group.Although irrigants agitation by an Er: Yag laser significantly minimized micro-hardness reduction, it did not suppress the adverse effects on dentine micro-hardness when two-irrigants were used.

31. Unnikrishnan M, Mathai V, Sadasiva K, Santakumari RSM, Girish S, Shailajakumari AK.(2019) [(38)] The aim of the study was to compare the effect of smear layer removal by 17% Ethylene diamine tetraacetic acid (EDTA), 17% Ethylene glycol tetraacetic acid (EGTA),10% Citric acid and (MTAD solution) a Mixture of tetracycline isomer, an acid and a detergent applied as final rinse, when used along with 2.5% (NaOCl) Sodium hypochlorite and its effect on dentin microhardness. Sixty single-rooted human mandibular premolars with minimum curvature (<5°) were instrumented using rotary instrumentation till apical enlargement size 35 RaCe file 0.04 taper. During instrumentation, the canals were irrigated with 2-mL 2.5% NaOCl. After instrumentation, teeth were rinsed with distilled water and were divided into 5 groups (n = 12) according to final rinse for 1 min with 5-mL 17% EDTA followed by 5-mL 2.5% NaOCl (group 1, control group), 17% EGTA (group 2), MTAD solution (group 3), 10% citric acid (group 4), and 17% EDTA (group 5). Teeth were split through the groove prepared, and one-half of specimen were evaluated for smear layer removal using scanning electron microscopy**.**Irrigation regimen following the use of 2.5% NaOCl during instrumentation followed by application of 5-mL 17% EDTA solution for 1 min resulted in efficient smear layer removal and less decrease in dentin microhardness compared with 17% EGTA, 10% citric acid, and MTAD solution.

32. Dhawan R, Gupta A, Dhillon JS, Dhawan S, Sharma T, Batra D.(2020) [39] The present *in vitro* study was undertaken to check the effect of the different irrigating solutions with surfactants, i.e., sodium hypochlorite-(Naocl)-Extra, chlorhexidine (CHX)-Ultra, ethylenediaminetetraacetic acid (EDTA), QMix, and BioPure MTAD on the microhardness and smear layer removal of root canal dentin.A total of 120 straight rooted lower premolars were collected and were randomly divided into 2 equal groups of 60 each (n = 60). The microhardness of the samples was evaluated by Vickers hardness tester and the removal of smear layer by scanning electron microscope after irrigation of the samples with the tested solutions.CHX-Ultra showed the least microhardness reduction, and EDTA showed the maximum microhardness reduction in all the tested groups. BioPure MTAD showed the maximum removal of smear layer in the apical third, and CHX-Ultra showed the minimal smear layer removal in the apical third.During smear layer removal, irrigating solutions cause alterations in the chemical composition of dentin, which may decrease the microhardness of the root dentin causing erosion and affecting the clinical performance of the endodontically treated teeth. Irrigating solution with maximum smear layer removal with minimum changes in microhardness should be used.

33. Pinto L, Cardoso I, Rover G, Bortoluzzi E, Garcia L, Teixeira CS. (2020) [10] evaluated the effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin. There were three groups according to the different irrigation protocols: 1% NaOCl; 1% NaOCl + 17% EDTA; and 5% NaOCl. After irrigation, the hardness measurement was done. Then, Ca(OH)2 dressing was applied and left for 30 days, until it was removed and a new microhardness measurement was made, in a third quadrant. There was a significant decrease of dentin microhardness after the irrigation protocols ,however, with no significant dif ference after Ca(OH)2 dressing .The 5% NaOCl group shown the greatest difference. All irrigation protocols promoted significant decrease of the dentin microhardness. The Ca(OH)2 dressing for 30 days did not significantly affect the microhardness of the root canal dentin.

34. Barbosa AFS, Mello LM, Teixeira FG, Rached-Júnior FJA, Trindade TF, Raucci-Neto W.(2020) [40]This study evaluated the effect of 17% EDTA, 10% citric acid (CA), and 2% chlorhexidine (CHX) activated with a rotary microbrush (CanalBrush) on root dentin microhardness, roughness, and epoxy-based sealer bond strength. One hundred sixty single-rooted bovine incisors were instrumented and divided into 8 groups according to treatment: 1. 17% EDTA; 2. 17% EDTA+2% CHX; 3. 10% CA; 4. 10% CA+2% CHX; 5. 17% EDTA with CanalBrush; 6. 17% EDTA+2% CHX with CanalBrush; 7. 10% CA with CanalBrush; and 8. 10% CA+2% CHX with CanalBrush. Ten roots in each group were split into halves and submitted to microhardness and roughness analyses ($n = 10$). Following endodontic filling with AH Plus sealer, 10 roots in each group underwent push-out bond strength testing ($n = 10$). All groups had similar microhardness values ($p > 0.05$) which was higher in the apical third than in the middle and cervical thirds ($p < 0.05$). The CanalBrush groups had higher roughness than the no-activation groups ($p < 0.05$), with significantly higher roughness in the cervical third than in the apical third ($p < 0.05$). All groups exhibited similar bond strength ($p > 0.05$), with the cervical third being higher, followed by the middle and apical thirds ($p < 0.05$). Microbrush activation had a direct impact on dentin roughness and did not influence the dentin microhardness or the retention of epoxy-based sealer to the root canal.

35. Durigon M, Cecchin D, de Carli JP, Souza MA, Farina AP. (2020) [41] evaluated the effect of different endodontic irrigation protocols on dentin mechanical properties and fracture resistance of roots with 0.5 mm (weakened roots) and 1.5 mm of thickness. Irrigation protocols were the following: Distilled water (DW) + Ethylenediamine tetraacetic acid (EDTA); grape seed extract (GSE) + EDTA; sodium hypochlorite (NaOCl) + EDTA; NaOCl + EDTA + GSE; calcium hypochlorite (Ca(ClO)2) + EDTA; Ca(ClO)2 + EDTA + GSE; chlorhexidine (CHX) + EDTA; CHX + EDTA + GSE. The samples were prepared and the values of microhardness, ultimate tensile strength (UTS), and flexural strength were obtained. Further, fracture resistance of roots with dentin thickness of 0.5 mm and 1.5 mm, and restored with fiberglass post relined with composite resin and metal crowns, were evaluated with same irrigation protocols previously described; the failure mode was evaluated as well. Higher reduction of dentin microhardness was observed in the NaOCl and NaOCl + EDTA + GSE groups ($p < 0.0001$). An increased in the UTS values was obtained in the CHX groups ($p < 0.0001$), while similar values were observed between the control and other groups ($p > 0.05$). The reduction of dentin flexural strength was observed in the NaOCl groups ($p < 0.0001$), while no significant changes were observed in the other groups ($p > 0.05$).An increased rate of irreparable failure was obtained in the NaOCl groups, whereas there was a predominance of repairable failure in the other groups.The endodontic irrigation protocol has a significant impact on the dentin mechanical properties; on the other hand, do not reduce the fracture resistance of root with 0.5 mm and 1.5 mm of thickness.

36. Bosaid F, Aksel H, Makowka S, Azim AA.(2020) [(42)] evaluated the effects of the prolonged use of various irrigant solutions used during regenerative endodontic procedures on the physical and chemical structure of root canal dentine in extracted human teeth. Sixty root dentine samples from extracted, single-rooted, human teeth were assigned to 10 groups. Eight groups were irrigated with 1.5% NaOCl for 5 min, followed by 3%, 10%, 17% EDTA or 10% citric acid (CA) for 5 or 10 min. One group received only NaOCl irrigation, and samples with only distilled water irrigation were used as a control group. The changes in microhardness and flexural strength were determined using Vickers and 3-point flexural tester, respectively. The application of 1.5% NaOCl for 5 min did not affect the mineral content or microhardness of dentine ($P > 0.05$). However, it significantly decreased the dentine collagen peak values ($P < 0.05$), which was similar to the control group after the use of chelating agents (EDTA and 10% of CA) ($P > 0.05$). The effect of EDTA on the inorganic content was not concentration and time dependent ($P > 0.05$). CA resulted in a significantly greater reduction in the inorganic contents compared with the control and EDTA groups ($P < 0.05$). Regardless of the time, EDTA and CA significantly decreased dentine microhardness compared to the control ($P < 0.05$) with the greatest reduction in the CA groups ($P < 0.05$). NaOCl alone was associated with the lowest flexural strength, while none of the other irrigation regimens significantly decreased the flexural strength compared to the control group ($P> 0.05$).Use of 1.5% NaOCl for 5 min decreased the collagen content of samples of human dentine from extracted teeth while EDTA and 10% citric acid mostly affected the inorganic content and microhardness of dentine surfaces. None of the irrigant solutions significantly decreased the mechanical properties of the entire dentine specimen.

37. Sahebi S, Sobhnamayan F, Moazami F, Naseri M.(2020) [43] This study aimed to evaluate the ability of sodium thiosulfate (STS) to neutralize the adverse effect of sodium hypochlorite (NaOCl) on dentin micro-hardness. Fifty single-rooted teeth were longitudinally sectioned. The samples divided into a control and four sample groups (n = 20). All the samples were immersed in different solutions as follows, Control: Normal saline for 15 min, G1and G2: 2.5% NaOCl for 15 min, G3: 2.5% NaOCl for 15 min, followed by 5% STS for 10 min, G4: Normal saline for 15 min followed by 5% STS for 10 min. All groups except G1 incubated for one week before the test. The micro-hardness of samples was measured. Data were analyzed using the Kruskal-Wallis test for pairwise comparisons. A p value < 0.05 was considered significant.All groups showed a significant decrease in the micro-hardness value compared with the control group. NaOCl for one week (G2) reduced the micro-hardness of dentine compared with samples, tested immediately after immersion in NaOCl (G1) ($p < 0.05$). NaOCl alone (G2) or treated with STS (G3) resulted in a significant decrease in micro-hardness compared with the STS group (G4) ($p < 0.05$).STS as a neutralizing agent could not prevent the dentin micro-hardness downturn caused by NaOCl.

38. Tsenova-Ilieva I, Karova E.(2021) [7] evaluated the effect of different irrigation regimens on root dentin microhardness. Twenty extracted, single rooted, non-endodontically treated upper incisors were decoronated and further sectioned longitudinally in buccolingual direction and were distributed into four groups and then immersed in the following solutions: group 1: 2% sodium hypochlorite for 2 minutes followed by 17% EDTA for 2 minutes; group 2: 2% sodium hypochlorite for 2 minutes followed by 17% EDTA for 2 minutes, both ultrasonically activated; group 3: 5.25% sodium hypochlorite for 2 minutes followed by 17% EDTA for 2 minutes; group 4: distilled water (control group). Afterwards, the samples were dried and subjected to microhardness testing .All irrigation regimens significantly decreased the mean hardness of dentin when compared to the control group 4 ($p<0.05$). It was concluded that the dentin microhardness was decreased regardless of the irrigation protocols used.

39. Kour S, Malik A, Choudhary A.(2021) [44] Irrigation solutions used and time of use has a definite effect on the micro hardness & other physical properties of dentin which in turn have direct consequence on the longevity functional performance of root canal treated teeth.Aim of the study was to evaluate the effect of different irrigation solutions on micro hardness of root dentin.Forty extracted single rooted lower premolars were used. After instrumentation all the root halves were randomly assigned into 4groups (n=10) and brought in contact with one of the following irrigants for 5 minutes.Group I: 10 ml of 5% Sodium Hypochlorite (NaOCl).Group II: 10 ml of 17% ethylene diamine tetra-acetic acid (EDTA) followed by 10 ml of 5% NaOCl.Group III: 10 ml of 5% NaOCl followed by 10 ml of 2% chlorhexidine digluconate (CHX).Group IV: 10 ml of 5% NaOCL followed by flush of 10 ml distilled water then by 10ml of 2% CHX.Dentin micro hardness was measured at baseline and after treatment to determine the change in micro hardness, using Vickers tester. EDTA with NaOCl causes greatest changes in dentine micro hardness, an intermediate flush with normal saline should be given for prevention of precipitation with NaOCl & CHX.

40. Elika V, Kunam D, Anumula L, Chinni SK, Govula K.(2021) [45] aimed to compare and evaluate the effect of Chloroquick with composition of 18% etidronic acid+ 5% sodium hypochlorite (NaOCl) with other irrigants such as Triphala, NaOCl, and ethylenediaminetetraacetic acid (EDTA) on the microhardness of root canal dentin. Forty freshly extracted non-carious single-rooted human teeth were collected and decoronated at CEJ to standardize the canal length. The roots were sectioned longitudinally to get two halves. Baseline microhardness evaluation was done using

Vickers microhardness test before the immersion in irrigants; samples were then randomly divided into four groups (*n*=20), based on the irrigant used as follows: Group 1 - Saline; Group 2 - 5% NaOCl +17% EDTA; Group 3 - Triphala; and Group 4 - Chloroquick. Later, the samples were immersed in the irrigating solutions for 15 min at 37°C for each group and were then subjected to post-treatment microhardness testing. Microhardness values were recorded and statistically analyzed using one-way ANOVA and intergroup comparison with *post hoc* Tukey test (P<0.05).The results of the present study showed that all the tested specimens showed a decrease in the microhardness values following application of different irrigating solutions except the control group. The use of Triphala and Chloroquick has minimal effect on the microhardness of root canal dentin post-treatment when compared with 5% NaOCl and 17% EDTA. It was concluded that Chloroquick, as well as 0.005% Triphala, can be used safely as an irrigating solution with less detrimental effects on the hardness of root dentin.

41. Arul B, Suresh N, Sivarajan R, Natanasabapathy V.(2021) [(46)] assessed the effect of volume of endodontic irrigants used in different final irrigation activation techniques on root canal dentin microhardness (RCDM).Sixty human maxillary central incisors were embedded in acrylic resin in Kuttler's endodontic cube to the level of cementoenamel junction. The root samples were randomly divided into 4 experimental groups (n = 15): Group-NI-needle irrigation, Group-PUI-continuous passive ultrasonic irrigation, Group-EndoVac-apical negative pressure system, Group combination- EndoVac + PUI irrigation. Root canals were instrumented up to size 40 (F4). The resin mounted specimens were sectioned longitudinally into two halves and were reassembled in Kuttler's kube to carry out final irrigation activation. A predetermined standardized volume of irrigants was used in each group. The RCDM was measured after root canal instrumentation and after final irrigation using Vicker microhardness tester (coronal, middle, and apical third). Reduction in RCDM was observed with all the endodontic irrigating techniques tested. EndoVac and combination irrigation techniques showed maximum reduction in RCDM in all thirds of root canal.

42. Philip PM, Sindhu J, Poornima M, Naveen DN, Nirupama DN, Nainan MT.(2021) [(47)] The aim of this *in vitro* study was to compare the effects of herbal irrigants with conventional irrigants on microhardness and flexural strength of root dentin. Sixty extracted permanent maxillary canines were selected. Decoronated roots were sectioned longitudinally into buccal and lingual segments to get 120 specimens. These were embedded in auto polymerizing acrylic resin and further grounded with fine emery papers under distilled water. Of these, 100 root segments without any defects were selected, further divided into four test groups and a control group according to the irrigants used (n = 20). Group 1: 2.5% Sodium hypochlorite, Group 2: Miswak stick extract, Group 3: Cashew leaves extract. Group 4: Mango leaves extract and Group 5: Normal saline (control). All specimens were treated with 5 ml of each irrigant for 10 minutes and rinsed immediately. Dentin microhardness was measured with a Vickers indenter, and the flexural strength test was done using a universal testing machine. The data were analyzed using one-way ANOVA and the intergroup comparison by student *t*-test.The experimental groups showed a significant reduction in microhardness values when compared with the control group. When compared to the control group, the flexural strength values decreased significantly with experimental groups.Within the limitation of this study, it was concluded that herbal irrigants were least detrimental to root dentin microhardness when compared with conventional irrigant. But the flexural strength was equally reduced by both conventional and herbal irrigants.

43. Kulkarni S et al (2021) [(48)] The aim of this study was to evaluate the effects of 2% chlorhexidine gluconate (CHG) and 2% sodium fluoride (NaF) as endodontic irrigants on microhardness of root dentin. In this *in vitro* study, access cavity and root canal preparations were done on 24 freshly extracted anterior teeth. After sectioning into 24 dentin discs using hard tissue microtome in 2 mm thickness, all samples were immersed in solutions of 17% ethylenediamine tetra-acetic acid (EDTA) (2 minutes) followed by 2.5% of sodium hypochlorite (NaOCl) (10 minutes). Then samples were randomly divided into three groups based on the irrigant used: Group I: saline (control group); Group II: 2% NaF; Group III: 2% CHG for two minutes each. Dentin microhardness was measured before (pretreatment), during (after treatment with 17% EDTA and 2.5% NaOCl), and after the experimental period (after treatment with saline, 2% NaF, and 2% CHG) using a Vickers indenter. Specimens rinsed in 2% CHG showed a significant increase in Vickers hardness number (VHN) values ($p <$ 0.05), as compared with EDTA and NaOCl groups, whereas saline and 2% NaF groups showed no significant difference..NaF did not show any significant effect on microhardness of the root dentin. CHG as an irrigant was seen to have a strengthening effect on dentin microhardness in comparison to NaOCl and EDTA, which has decreased the strength of root dentin.

MATERIALS
&
METHODS

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Praj Mettallurgical Laboratory, Pune.

A total of 95 human permanent maxillary central incisor teeth were collected following the inclusion and exclusion criteria. Teeth were cleaned with scalers for any tissue remnants, plaque and calculus on the roots.

The following inclusion & exclusion criteria were set to select the teeth:

INCLUSION CRITERIA

Completely developed permanent maxillary central incisors having single root with one canal and minimum curvature.

EXCLUSION CRITERIA

1. Teeth with any crack ,caries or calcification.
2. Teeth with any developmental anomaly.
3. Teeth with any previous restoration.
4. Endodontically treated teeth.

STUDY SAMPLE AND SIZE

The sample size was calculated according to the formula below:

$$N = \frac{[Z_{a/2} + Z_b)^2 \times \{2 (sd)^2\}]}{(\text{Effect size})^2}$$

where,

Z_a , Z_b - are the alpha and beta values taken from the table of probabilities of the standard normal distribution for the desired confidence level.

sd – standard deviation

Effect size – is the number measuring the strength of the relationship between two variables in a population, or a sample based estimate of that quantity.

The key article was analysed to determine the sample size. Calculations were made. A standard deviation of 3.9343 was considered. Effect size of 5.3 was obtained and Za level at 5% significance was taken for calculation

It was finalized that , a total of 19 samples were required in each group.

Altogether, for the 5 tested groups, a sample size of 95 (19 X 5) was taken for the research purpose.

Table A : MATERIALS AND ARMAMENTARIUM USED FOR SAMPLE PREPARATION:

S. No.	Material & Armamentarium	Manufacturer
1.	Ultrasonic Scaler with tips	Coltene, Switzerland
2.	Straight hand piece	Marathon, Korea
3.	Micro motor (Slow Speed)	Unicorn Denmart, India
4.	Diamond disc & Mandrel	Horico, Germany
5.	K-files (ISO #6,8,10,15,20)	Dentsply, U.S.A.
6.	Rotary HyFlex CM files (4% 20 & 4% 25)	Coltene, Switzerland
7.	Endo block	Dentsply, U.S.A.
8.	X ray film	Avue, India
9.	4% Formalin	Fizmerk India Chemicals, India
10.	Distilled Water	Fizmerk India Chemicals, India
11.	Surgical Chisel	Henry Schein, USA
12.	Aluminium oxide paste	Cosmedent, Canada
13.	Silicon carbide abrasive paper	Polar star, India
14.	Cyanoacrylate Adhesive	Evobond, Taiwan

Table B : IRRIGATING SOLUTIONS TO BE TESTED :

S. No.	Material & Armamentarium	Manufacturer
1	3 % sodium hypochlorite (NaOCl)	Pyrax, India
2	Chlorhexidine 2 %	PrevestDenpro, India
3	5% Calcium Hypochlorite	Gyan Scientific Traders, India
4	Chitosan 0.2 Wt%	Nano wings, India
5	Normal Saline (0.9%w/v NaCl)	KRPL, India
6	Disposable syringe of 5ml with 27 gauge needle	Dispo Van, India
7	Absorbent Paper Points (#25 - #35)	Diadent,India
8	Root canal Irrigation Needle(With side vents) – 26 Guage	Vishal Dentocare Pvt Limited,India
9	Disposable syringe of 5ml with 26 gauge needle	Dispo Van, India

METHODOLOGY

SAMPLE COLLECTION

Ninety five freshly extracted maxillary central incisor teeth were obtained from Department of Oral and Maxillofacial Surgery, Babu Banarasi Das College Of Dental Sciences, Lucknow. Teeth were cleaned mechanically for any tissue remnants, plaque and calculus on the roots. Later teeth were examined clinically and radiographically to follow inclusion and exclusion criteria. The extracted teeth were then stored in 4% formalin for 72 hours after which the specimen preparation was initiated.

SPECIMEN PREPARATION

Teeth were sectioned transversely at cementoenamel junction using diamond disc operated by low speed hand piece under continuous water coolant. Patency of the root canal was achieved using size 10 K file. The working length was established by introducing a K-file #10 in the canal until its tip was visualized at the apical foramen. A nickel-titanium rotary system (Hyflex CM,Coltene) was employed for shaping the canal using crown down technique. The canals were shaped to a final size of 4/25. During preparation, the root canals were irrigated with 2 mL of distilled water using 26 guage side vented needle. A final irrigation with 20 ml distilled water was performed for the removal of possible dentin chips. Root canals were dried with appropriate absorbent paper points. After canal preparation the roots were sectioned longitudinally. For this, grooves were prepared along the long axes of the roots. This was done using a water cooled diamond disk mounted on a high speed handpiece. A surgical chisel was then used to cleave the roots in a buccolingual direction. The resulting specimens were ground and polished with silicon carbide abrasive papers under normal saline to remove any surface scratches and were fixed in resin acrylic blocks using cyanoacrylate adhesive. The acrylic block sets were sorted into five groups, depending on the irrigating solution being used. Before irrigating the root canal lumen with the test substances, dentin surface was polished with felt discs embedded in aluminum oxide paste at a low speed.

Table C : GROUPWISE DISTRIBUTION OF SAMPLES

GROUPS	NO. OF SAMPLES	TEST IRRIGANTS
GROUP A	19	Normal Saline
GROUP B	19	3% sodium hypochlorite
GROUP C	19	2% chlorhexidine digluconate
GROUP D	19	5% Calcium hypochlorite
GROUP E	19	0.2% Nano Chitosan

IRRIGATION OF SAMPLES

Irrigation of samples in Group A : A standardized volume of 1ml of normal saline was delivered directly on root canal dentin using a 27 gauge needle. This was repeated by delivering another 1ml of normal saline after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was again delivered. So after 15 minutes a total of 3ml of normal saline was delivered. Finally the specimens were rinsed with 20 mL of distilled water to remove any residues of the test solution. The specimens were then blotted dry to be submitted for dentin microhardness evaluation.

Irrigation of samples in Group B : Using a 27 guage needle, 1ml of 3% sodium hypochlorite was delivered on the root dentin surface. This was repeated by delivering another 1ml of 3% sodium hypochlorite after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was delivered. So after 15 minutes a total of 3ml of 3% sodium hypochlorite was delivered. To remove any residues of the test irrigant, after 15 minutes, the specimens were rinsed with 20 ml of distilled water. The specimens are then made ready for dentin microhardness evaluation , after drying the samples.

Irrigation of samples in group C : 1ml of 2% chlorhexidine digluconate was taken and delivered directly on the dentin surface of the specimen using a 27 guage needle. This was repeated by delivering another 1ml of 2% chlorhexidine digluconate after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was delivered. So after 15 minutes a total of 3ml of 2% chlorhexidine digluconate was delivered. After 15 minutes, the specimens were rinsed with 20 mL of distilled water to remove any residues of the test solution. The specimens were then blotted dry to be submitted for dentin microhardness evaluation.

Irrigation of samples in group D : A standardized volume of 1ml of 5% calcium hypochlorite solution was carefully measured and delivered to the root dentin surface using a 27 guage needle. This was repeated by delivering another 1ml of 5% calcium hypochlorite solution after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was delivered. So after 15 minutes a total of 3ml of 5% calcium hypochlorite was delivered. The specimens were rinsed with 20 mL of distilled water to remove any residues of the test solution. The specimens were then blotted dry to be submitted for dentin microhardness evaluation.

Irrigation of samples in group E : Using a 27 guage needle, 1ml of 0.2 % Nano Chitosan solution was delivered on the root dentin surface. This was repeated by delivering another 1ml of 0.2 % Nano Chitosan solution after a time period of 5 minutes. Finally after 5 more minutes, 1ml of the irrigant was delivered. So after 15 minutes a total of 3ml of 0.2 % Nano Chitosan solution was delivered.The specimens were rinsed with 20 mL of distilled water to remove any residues of the test solution. The specimens were then blotted dry to be submitted for dentin microhardness evaluation.

ANALYSIS OF THE SAMPLE

DENTIN MICROHARDNESS EVALUATION

The dentin microhardness was measured with the Vicker's microhardness tester. In each sample, three indentations were made along lines parallel to the edge of the root canal lumen. At each measurement, 1 indentation was made using a 50-g load perpendicular to the indentation surface with a dwell time of 10 seconds. The first indentation was made 1000 micrometer from the root canal entrance and two other indentations were made at a distance of 200 micrometer from each other. The hardness values for each specimen was obtained as the average of the results for the three indentations.

Raw data were obtained and sent for statistical analysis.

Fig 1 – Teeth collected for study

Fig 2 – Armamentarium for specimen preparation

Fig 3 – Decoronation of sample teeth

Fig 4 – Armamentarium for root canal preparation

Fig 5 – Cleaning and shaping

Fig 6 – Longitudinal sectioning of tooth

Fig 7 Mounted tooth section

Fig 8 – Normal saline

Fig 9 – 3 % Sodium Hypochlorite

Fig 10 – 2% Chlorhexidine Digluconate

Fig 11 – Calcium Hypochlorite

Fig 12 – 0.2% Chitosan nanoparticle

Fig 13- Armamentarium for irrigation

Fig 14 – Vicker's Microhardness tester

Fig 15 – Testing for Microhardness

OBSERVATION
&
RESULTS

Descriptive and analytical statistics were done.The normality of data was analyzed by the Shapiro-Wilktest. As the data followed normal distribution the parametric tests were used to analyze the data. The one-way analysis of variance (ANOVA) test was used to check mean differences among the groups. Post hoc analysis was done using Tukey's HSD test. **Software:** SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA).

Table 1: Comparison of mean microhardness (HV)among the groups

Groups	N	Mean	S.D.	S.E.	Min.	Max.	F-value	P-value[#]
Group A	19	58.43	2.61	0.59	53.58	62.34	230.168	<0.001[†]
Group B	19	47.07	1.04	0.23	45.38	48.90		
Group C	19	47.39	1.93	0.44	44.59	51.20		
Group D	19	42.43	1.62	0.37	39.90	45.31		
Group E	19	42.62	1.76	0.40	40.11	45.80		

[#]P-value derived from one-way ANOVA test; [†]significant at $p < 0.05$

The mean microhardness (HV)was compared among the five groups. The analysis done by one-way ANOVA showed statistically significant differences (**p<0.001**) in mean microhardness (HV). The group A– Saline group had the highest microhardness (HV)of 58.43± 2.61 followed by group C- 0.2% Chlorhexidine group(47.39± 1.93), group B- Sodium Hypochlorite group (47.07± 1.04) and group E- Chitosan group (42.62± 1.76). The group D - 5% Calcium Hypochlorite group had the lowest microhardness (HV) of 42.43±1.62.

Table 2: Post hoc pair wise comparison of mean microhardness (HV)between group A and other groups

Groups	**M.D.**	**95% C.I.**	**P-value***
Group A v/s Group B	11.35	9.66-13.04	<0.001†
Group A v/s Group C	11.03	9.35-12.72	<0.001†
Group A v/s Group D	15.99	14.30-17.67	<0.001†
Group A v/s Group E	15.80	14.11-17.48	<0.001†

#P-value derived from Tukey's HSD post hoc test; †significant at p < 0.05

The post hoc pair wise comparative analysis was done. When group A was compared with group B, a mean difference of 11.35(95% CI: 9.66-13.04) was seen which was statistically significant (**p<0.001**). When group A was compared with group C, a mean difference of 11.03(95% CI: 9.35-12.72) was seen which was statistically significant (**p<0.001**). When group A was compared with group D, a mean difference of 15.99(95% CI: 14.30-17.67) was seen which was statistically significant (**p<0.001**). When group A was compared with group E, a mean difference of 15.80(95% CI: 14.11-17.48) was seen which was statistically significant (**p<0.001**).

Table 3: Post hocpair wise comparison of mean microhardness (HV)between group B and other groups

Groups	M.D.	95% C.I.	P-value[*]
Group B v/s Group A	-11.35	-13.04--9.66	<0.001[†]
Group B v/s Group C	-0.31	-2.00-1.37	0.985
Group B v/s Group D	4.63	2.94-6.32	<0.001[†]
Group B v/s Group E	4.44	2.76-6.13	<0.001[†]

[#]P-value derived from Tukey's HSD post hoc test; [†]significant at $p < 0.05$

The post hoc pair wise comparative analysis was done. When group B was compared with group A, a mean difference of 11.35 (95% CI: 9.66-13.04) was seen which was statistically significant (**p<0.001**). When group B was compared with group C, a mean difference of -0.31(95% CI: -2.00-1.37) was seen which was**NOT**significant (**p=0.985**). When group B was compared with group D, a mean difference of 4.63(95% CI: 2.94-6.32) was seen which was statistically significant (**p<0.001**). When group B was compared with group E, a mean difference of 4.44(95% CI: 2.76-6.13) was seen which was statistically significant (**p<0.001**).

Table 4: Post hocpair wise comparison of mean microhardness (HV)between group C and other groups

Groups	**M.D.**	**95% C.I.**	**P-value***
Group C v/s Group A	-11.03	-12.72--9.35	<0.001†
Group C v/s Group B	0.31	-1.37-2.00	0.985
Group C v/s Group D	4.95	3.26-6.63	<0.001†
Group C v/s Group E	4.76	3.07-6.44	<0.001†

#P-value derived from Tukey's HSD post hoc test; †significant at $p < 0.05$

The post hoc pair wise comparative analysis was done. When group C was compared with group A, a mean difference of -11.03(95% CI: -12.72--9.35) was seen which was statistically significant (**p<0.001**). When group C was compared with group B, a mean difference of 0.31 (95% CI: -1.37-2.00) was seen which was**NOT**significant (**p=0.985**). When group C was compared with group D, a mean difference of 4.95(95% CI: 3.26-6.63) was seen which was statistically significant (**p<0.001**). When group C was compared with group E, a mean difference of 4.76(95% CI: 3.07-6.44) was seen which was statistically significant (**p<0.001**).

Table 5: Post hocpair wise comparison ofmean microhardness (HV)between group D and other groups

Groups	**M.D.**	**95% C.I.**	**P-value**[*]
Group D v/s Group A	-15.99	-17.67--14.30	<0.001[†]
Group D v/s Group B	-4.63	-6.32--2.94	<0.001[†]
Group D v/s Group C	-4.95	-6.63--3.26	<0.001[†]
Group D v/s Group E	-0.18	-1.87-1.49	0.998

[#]P-value derived from Tukey's HSD post hoc test; [†]significant at $p < 0.05$

The post hoc pair wise comparative analysis was done. When group D was compared with group A, a mean difference of -15.99(95% CI: -17.67--14.30) was seen which was statistically significant (**p<0.001**). When group D was compared with group B, a mean difference of -4.63(95% CI: -6.32--2.94) was seen which wasstatistically significant (**p=0.985**). When group D was compared with group C, a mean difference of -4.95(95% CI: -6.63--3.26) was seen which was statistically significant (**p<0.001**). When group D was compared with group E, a mean difference of -0.18(95% CI: -1.87-1.49) was seen which was **NOT**statistically significant (**p=0.998**).

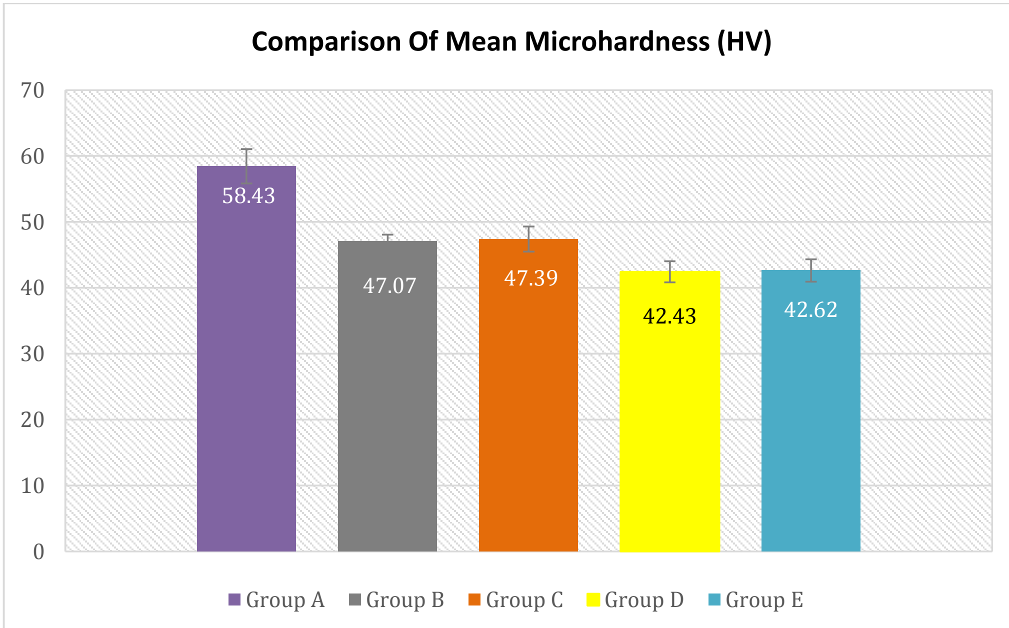
Table 6: Post hocpair wise comparison ofmean microhardness (HV) between group E and other groups

Groups	**M.D.**	**95% C.I.**	**P-value[*]**
Group D v/s Group A	-15.80	-17.48--14.11	<0.001[†]
Group D v/s Group B	-4.44	-6.13--2.76	<0.001[†]
Group D v/s Group C	-4.76	-6.44--3.07	<0.001[†]
Group D v/s Group E	0.18	-1.49-1.87	0.998

[#]P-value derived from Tukey's HSD post hoc test; [†]significant at $p < 0.05$

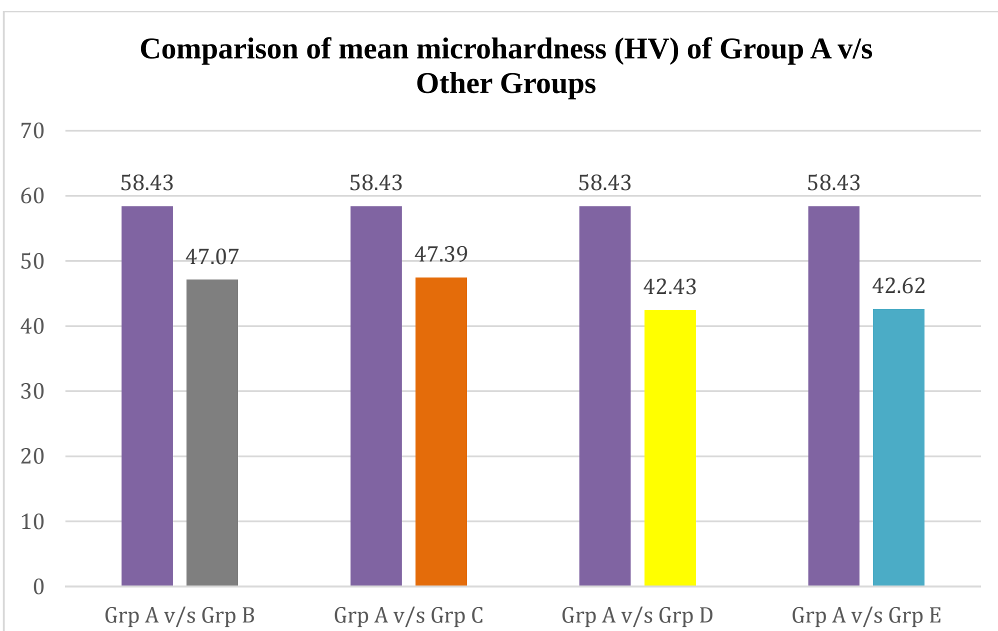
The post hoc pair wise comparative analysis was done. When group E was compared with group A, a mean difference of -15.80(95% CI: -17.48--14.11) was seen which was statistically significant (**p<0.001**). When group E was compared with group B, a mean difference of -4.44(95% CI: -6.13--2.76) was seen which was significant (**p=0.985**). When group E was compared with group C, a mean difference of -4.76(95% CI: --6.44--3.07) was seen which was statistically significant (**p<0.001**). When group E was compared with group D, a mean difference of -0.18 (95% CI: -1.49-1.87) was seen which was statistically significant (**p=0.998**).

Graph 1: Comparison of mean microhardness (HV)among the groups

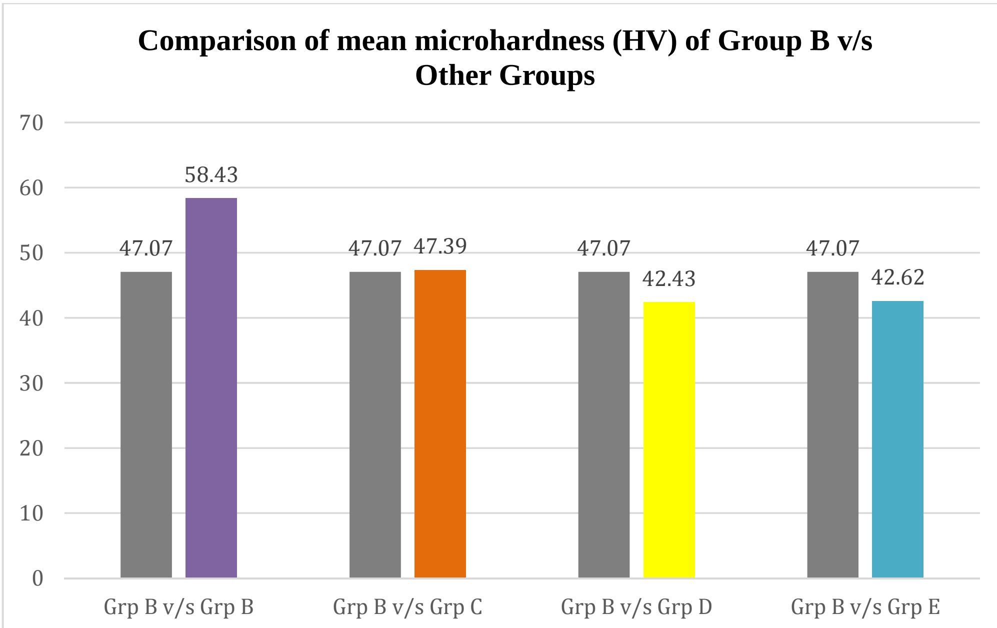


Note: The error bar represents standard deviation

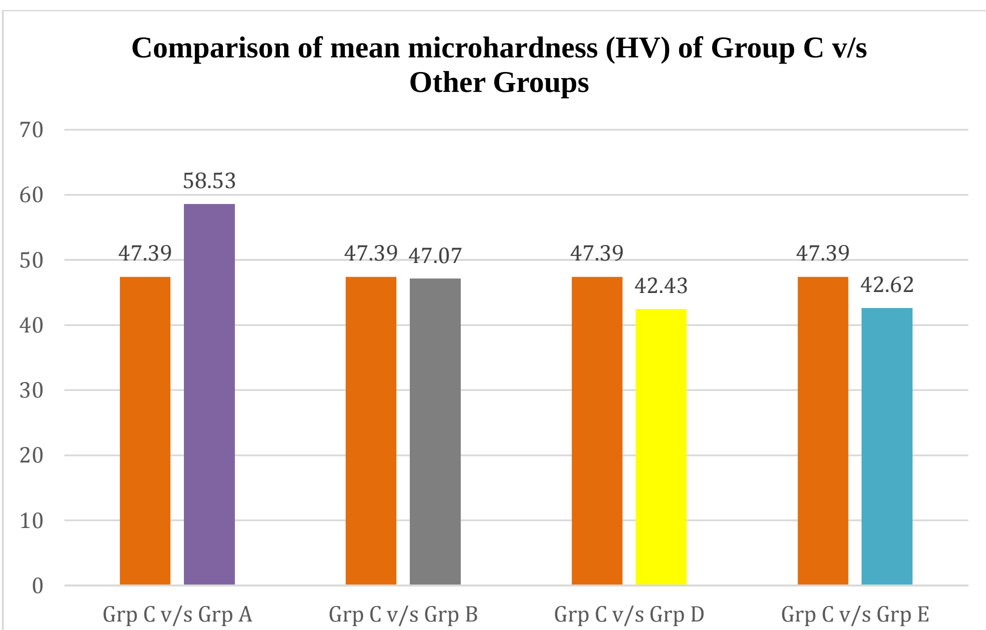
Graph 2: Comparison of mean microhardness (HV) of Group A v/s Other Groups



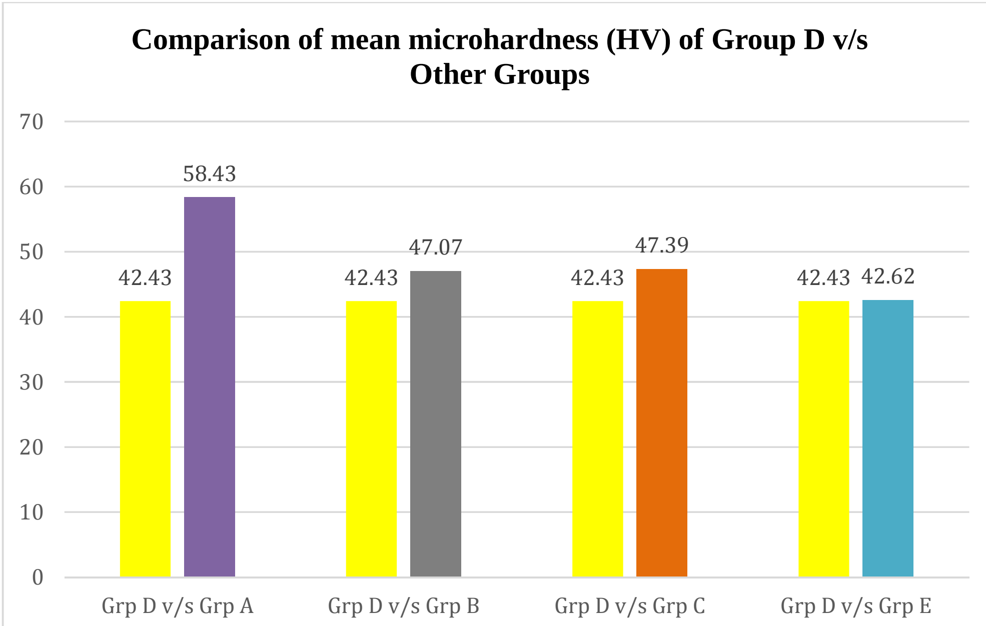
Graph 3: Comparison of mean microhardness (HV) of Group B v/s Other Groups



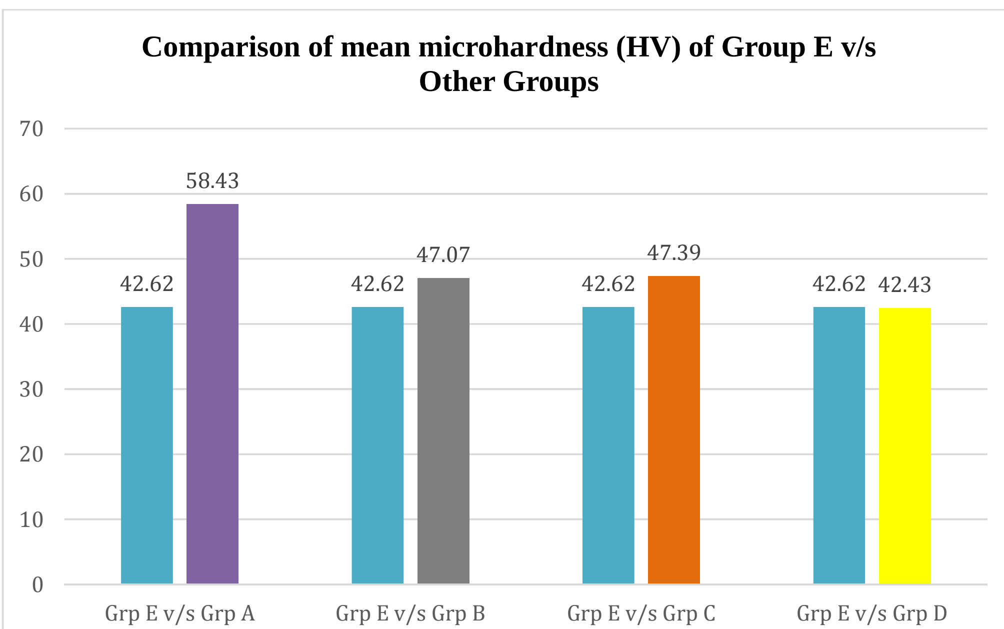
Graph 4: Comparison of mean microhardness (HV) of Group C v/s Other Groups



Graph 5: Comparison of mean microhardness (HV) of Group D v/s Other Groups



Graph 6: Comparison of mean microhardness (HV) of Group E v/s Other Groups



DISCUSSION

The present in-vitro study was conducted in the Department of Conservative Dentistry and Endodontics, Babu Banarasi Das College of Dental Sciences, Lucknow in collaboration with Praj Mettallurgical Lab, Pune.

The main objective of the chemomechanical preparation is to promote cleaning, shaping and disinfection of the root canal system. However, the complex anatomy of the root canal, which includes accessory canals, isthmuses, and apical ramifications, may hinders a proper disinfection. Therefore, the success of the endodontic treatment also depends on the chemical action of irrigating solutions. [10] An ideal irrigant should have these requisite functions: Lubrication, debridement, antimicrobial effect, and dissolution of organic and inorganic material. Unfortunately, there is no single irrigant to date that fulfills all these ideal requisites. [4]

Irrigation solutions during endodontic treatment may cause alterations in the chemical composition of den tin. The change of Calcium – Phosphorous ratio affects the original proportion of organic to inorganic components, which in turn changes the microhardness, solubility, permeability and surface roughness of dentin. These effects depend on the application time, the concentration of the irrigants and the irrigation protocol used throughout the endodontic treatment. [7]

These alterations in the chemical structure caused by the action of endodontic irrigants may in turn affect the mechanical properties of dentin. It consequently leads to the exposure of collagen and eventually causes decrease in dentin micro-hardness. Pashley D et al., suggested that an inverse relation exists between the dentin micro-hardness and density of the dentinal tubules. Reduced micro-hardness may lead to reduction in modulus of elasticity and flexural strength of dentin. [9] Hence the determination of micro-hardness provides an arbitrary assessment of the change in any mineral content of dental hard tissues.

Microhardness is considered as an indirect evidence of mineral changes in root canal dentin. [4] Microhardness is defined as the resistance to local deformation and is based on the induced permanent surface deformation that remains after removal of load. [1] Any change in the microhardness of the root dentin may adversely affect sealing ability and adhesion of dental material such as resin cements and root canal sealers to dentin, as well as inhibit resistance to bacterial ingress and permitting coronal leakage. [1] [8] It is known clinically that reduction of root dentin microhardness facilitates the introduction of instruments in cases of narrow canals, in addition to favoring the excision of dentin during the biomechanics of the teeth in general. [16] Microhardness tests are commonly used to study the physical properties of materials, and they are widely used to measure the hardness of teeth. This method is easy, quick, and requires only a tiny area of specimen surface for testing. Microhardness tests include Knoop Microhardness Test and Vickers Microhardness tests. [1] In our research,Vickers microhardness test was performed to evaluate the microhardness of root dentin. This test was chosen because it is less sensitive to surface conditions among the other microhardness measurement methods and more sensitive to measurement errors when equal loads are applied. [2] Studies done by Kour S et al (2021) [44], Tsenova et al (2021) [49] Duvvi SAB et al (2018) [4] have employed vickers's microhardness test to evaluate root dentin microhardness after irrigating with various irrigating solutions.

In our research, four different irrigating solutions were evaluated – 3% sodium hypochlorite (NaOCl) - Group B, 2% chlorhexidine gluconate (CHX) - Group C , 5% calcium hypochlorite solution ($CaOCl_2$) - Group D, and 0.2% chitosan nanoparticle solution – Group E. 3% sodium hypochlorite (NaOCl) irrigant was used as it is most commonly used endodontic irrigant used. It is employed in concentrations ranging from 0.5% to 6.25% . It is an antibacterial agent capable of dissolving vital and necrotic organic tissue, as well as the organic component of the smear layer. The degradation of the organic components by NaOCl solutions can alter adversely dentinal biomechanics by significantly decreasing its elastic modulus and flexural strength. [7] Cytotoxic activity is a well-known shortcoming of NaOCl that may cause acute injuring effects, if it reaches the periapical area. NaOCl extrusion during root canal therapy is commonly referred to as "hypochlorite accident;" it causes acute immediate symptoms and potentially serious sequelae. [10]Another irrigant tested was 2% chlorhexidine gluconate (CHX). Chlorhexidine (CHX) is a synthetic material comprising two biguanide groups and two symmetric 4-cholorophenyl rings, connected by a hexamethylene chain. Although CHX is useful as a final irrigant, its use as a main endodontic irrigant of the canal is not advised due to its inability to dissolve necrotic remnants and to the fact that it is less effective against gram-negative microorganisms than against gram-positive microorganisms. The principal challenge that prevents the use of CHX as a routine irrigant in endodontics is its lack of tissue solubility during chemomechanical preparation. [11]

In order to overcome all the drawbacks of the irrigants discussed above, there is a need to find a new effective and safer irrigant for the root canal treatment. 5% calcium hypochlorite solution ($CaOCl_2$) was used in this study. Calcium hypochlorite (Ca[OCl]2) is a white powder used for industrial sterilization, bleaching, and purifying water treatment. It is relatively stable and has greater available chlorine compared with NaOCl. Its incorporation in water can be more accurate than preparations by dilution of a more concentrated solution, which can be an advantage for clinical use. De Almeida et al. showed that Ca(OCl)2 associated with ultrasonic irrigation is efficient to reduce root canal contamination and can aid in chemomechanical preparation, and it was as effective as NaOCl [13] (4) Another newer irrigant tested was 0.2% chitosan nanoparticle solution. . Nanoparticles (NPs) as one of the novel strategies have been at the center of attention in the past few decades owing to their innovative and functional properties. In nanotechnology, a decrease in dimensions up to the atomic level leads to a considerable increase in surface area of the agent; therefore, the contact of NPs with the microorganisms and accordingly the effective interaction with the membrane of pathogen increases. In brief, high surface areas of NPs and consequently higher concentrations at target site are the most effective factors in antimicrobial behaviour when compared with their conventional counterparts. [15] Chitosan is a natural polysaccharide, prepared by the deacetylation of chitin, which is obtained from the shells of crabs and shrimps. This polysaccharide is endowed with properties of biocompatibility, biodegradibility, bioadhesion and atoxicity to human cells . It also presents low cost, in addition to a high chelating capacity for different metallic ions. [16]So in our research we evaluated the effect of two irrigants – sodium hypochlorite and chlorhexidine digluconate on root dentin microhardness and then that of two newer irrigants – calcium hypochlorite and chitosan nanoparticle solution.

In the present study, 95 human maxillary central incisors were taken into consideration after accomplishing the inclusion and exclusion criteria. Maxillary central incisors were taken for this research because they could be standardized because of their relative dimensions and similarity in morphology. [16] Another criteria that preferred central incisors for this research purpose was the ease of sectioning these single rooted teeth longitudinally. This in turn helped in exposing the root dentin surfaces microhardness measurement. [14]

The teeth were transversely sectioned at cemento enamel junction.This was done to obtain a uniform root length for all samples. [36] [50]

Hyflex CM rotary files were used for root canal preparation in the current study. These files have shown to produce lesser dentinal cracks during instrumentation and high cyclic fatigue resistance and hence were used. [51]

Hyflex files were employed in the crown down technique. The crown down approach was employed in the research as it has several advantages like early organic debris removal, the creation of a large reservoir for irrigating solutions and greater precision with regard to the exact working length and apical size. [52] The canals were shaped to a final size of 4/25 for sample preparation in this study. Minimum apical preparation size of #25 have been advocated for the prevention of iatrogenic instrumentation, like the occurrence of zips, or causage of apical transportation. [53] Also formation of apical cracks are minimal when size 25 rotary files are used. [54] A 0.04 taper will allow for tooth structure preservation and maximum volume of irrigation at apical third. [55] During canal preparation, the root canals were irrigated with 2 mL of distilled water using 26 guage side vented needle. A final irrigation with 20 ml distilled water was performed for the removal of possible dentin chips.This was done following the study design of Cruz Filho et al 10 where 1% sodium hypochlorite was used instead of distilled water. In this research, 1% sodium hypochlorite was not used since sodium hypochlorite was itself an irrigating solution whose effect on microhardness of root dentin was to be tested. Distilled water was used in our study for irrigation as well as for the removal of dentin chips since distilled water when used to irrigate the canal did not significantly change microhardness. [1] [8]

After canal preparation the roots were sectioned longitudinally using a water cooled diamond disk to cleave the roots in a buccolingual direction. The roots were sectioned longitudinally inorder to expose the root dentin surface, on which microhardness was to be measured in the later stage. [8] The specimens were then subjected to thorough polishing. They were first ground and polished with silicon carbide abrasive papers .. This was done to remove any surface scratches on the dentin surface that would be later subjected to microhardness testing. [8] [17] [23] The specimens were fixed in resin acrylic blocks using cyanoacrylate adhesive so that they could be mounted on the stage of Vicker's micro-hardness tester for testing to be done. (17) Later in the study the dentin surface was polished with felt discs embedded in aluminum oxide paste at a low speed.This was to remove the excess resin material present on the tooth surface. (14)

The group A– Saline group (control) had the highest microhardness (HV) of 58.43± 2.61 followed by group C- 2% Chlorhexidine group (47.39± 1.93), group B- Sodium Hypochlorite group (47.07± 1.04) and group E- Chitosan group (42.62± 1.76). The group D - 5% Calcium Hypochlorite group had the lowest microhardness (HV) of 42.43±1.62. (Table 1,Graph 1)

According to the Table No. 1, 5% Calcium Hypochlorite (Group D) demonstrated maximum reduction in microhardness. (42.43±1.62) (Table 1). This finding can be due to the ability of calcium hypochlorite to increase dentin permeability which may lead to greater sequestration of calcium ions,causing surface demineralization. (32) In a similar type of study done by Taffarel et al., Calcium hypochlorite demonstrated lowest reduction in microhardness amongst all the tested materials which is in accordance to present study.However in a study conducted by Dutta et al (25), it was found that the sodium hypochlorite showed more dissolution ability than calcium hypochlorite.

According to Table No. 1, after calcium hypochlorite, 0.2 % chitosan nanoparticle solution group showed the greatest reduction in microhardness. (42.62± 1.76) This finding may be due to the chelating action of chitosan. It is observed that adsorption and ionic exchange are responsible for the formation of complexes between chitosan and the metallic ions. Currently, there are two versions that try to explain the chelation process of chitosan. The first, known as the model of the bridge, is grounded in the theory that two or more amino groups of one chitosan chain will bind to the same metallic ion.According to the other version, only one amino group of chitosan is involved in the binding, that being the metallic ion "anchored" to the amino group (16) .In a similar type of study done by Saha et al (17)., chitosan group demonstrated lowest reduction in microhardness amongst all the tested materials except calcium hypochlorite which is in accordance to the present study. But according to a study done by Pimenta et al [16], chitosan did not significantly lower the microhardness values amongst the other tested agents.

When chitosan nanoparticle group was compared with group calcium hypochlorite group, a mean difference of -0.18 (95% CI: -1.49-1.87) was seen which was statistically significant (p=0.998). (Table 6, Graph 6) Calcium hypochlorite showed more reduction in microhardness than chitosan group.

According to the Table No. 1, after 0.2 % chitosan nanoparticle solution, 3% sodium hypochlorite group showed the greatest reduction in microhardness. (47.07± 1.04) This may be due to the organic dissolving properties of sodium hypochlorite on collagen component of dentin. In addition to that sodium hypochlorite extract the calcium ion from the dentin and decrease the calcium/ phosphorus ratio. [1] This property might be the cause for sodium hypochlorite to demonstrate a reduced microhardness value. This result is in accordance with studies done by Taffarel et al [2], Patil et al [14] and Ari et al [8] where sodium hypochlorite showed more reduction than the other tested samples , but lesser than that of calcium hypochlorite.

When sodium hypochlorite group was compared with group calcium hypochlorite group , a mean difference of 4.63(95% CI: 2.94-6.32) was seen which was statistically significant (p<0.001) (Table 3, Graph 3).Calcium hypochlorite group showed the least microhardness amongst the two. This finding is in accordance with similar studies done by Taffarel et al [2] and Duvvi et al [4] where calcium hypochlorite group showed more reduction in microhardness than sodium hypochlorite.

When sodium hypochlorite group was compared with 0.2% chitosan nanoparticle group, a mean difference of 4.44(95% CI: 2.76-6.13) was seen which was statistically significant (p<0.001). (Table 3, Graph 3). Chitosan group showed the least microhardness.This finding is in accordance with a similar study done by Saha et al [17] where the chitosan group showed more reduction in microhardness than sodium hypochlorite.

According to the Table No. 1, the 2% chlorhexidine digluconate group showed the least reduction in microhardness. (47.39± 1.93) In a similar type of study done by Taffarel et al. [2], chlorhexidine digluconate demonstrated the lowest microhardness amongst all the tested materials which is in accordance to present study.

When chlorhexidine digluconate group was compared with calcium hypochlorite group, a mean difference of 4.95(95% CI: 3.26-6.63) was seen which was statistically significant (p<0.001) (Table 4, Graph 4) Calcium hypochlorite showed more reduction in microhardness than chorhexidine group. This finding is in accordance with a similar study done by Taffarel et al [2] where calcium hypochlorite group showed more reduction in microhardness.

When chlorhexidine digluconate group was compared with 0.2 % chitosan nanoparticle group, a mean difference of 4.76(95% CI: 3.07-6.44) was seen which was statistically significant (**p<0.001**). (Table 4, Graph 4) The chitosan group showed more reduction in microhardness than chorhexidine group.

When chlorhexidine digluconate group was compared with sodium hypochlorite group , a mean difference of 0.31 (95% CI: -1.37-2.00) was seen which was not significant (p=0.985). (Table 4, Graph 4) Sodium hypochlorite showed more reduction in microhardness than chorhexidine group. This finding is inaccordance with similar studies done by Oliveira et al [5] , Aslantas et al [30], Bakr et al [33]Patil et al [14] and Ari et al [8] where sodium hypochlorite group showed more reduction in microhardness.

To conclude, calcium hypochlorite solution and chitosan nanoparticle solution showed the maximum reduction in microhardness than the other tested irrigants. Though low microhardness values favor easier instrumentation in narrow canals [16], a drop in microhardness values may adversely affect sealing ability and adhesion of dental material such as resin cements and root canal sealers to dentin, as well as inhibit resistance to bacterial ingress and permitting coronal leakage. [1] [8] Considering these facts it could be concluded that the conventional irrigant solutions – sodium hypochlorite and chlorhexidine digluconate may be continued to be used as a preferred irrigant over calcium hypochlorite and chitosan nanoparticle solution. Also further in vivo studies are required to test their effect in the clinical scenario.

CONCLUSION

Within the limitations of this in vitro study, it may be concluded that 5 % calcium hypochlorite solution reduced the root dentin microhardness the most among the tested irrigant solutions. 0.2% chitosan nanoparticle solution was the next irrigant which showed maximum reduction in microhardness followed by 3% sodium hypochlorite and 2% chlorhexidine digluconate.

From the study it is inferred that the newer irrigants – calcium hypochlorite and chitosan nanoparticle solution, though having some superior properties than their conventional counterparts, however they cannot fully replace conventional irrigants in routine endodontic practice due to their deleterious influence in the mechanical integrity of dentin as is evident by the drop in microhardness. Therefore further studies are required to test their effect in the clinical conditions.

REFERENCES

1. Abbas FS, Abdulredah NJ,Hassan AS.(2018) Effect of Final Irrigation Protocol on Dentin Microhardness. Biomedical & Pharmacology Journal, Vol. 11(4), p. 2157-2162.

2.Taffarel C, Bonatto FD,Bonfante FC,Palhano HS,Vidal CMP,Cecchin D,Souza MA.(2018)Effect of chemical and natural irrigant solutions on microhardness of root dentin – an in vitro study.Brazilian Journal of Oral Sciences,Volume 17.p.1-9.

3. Ari H, Erdemir A, Belli S.(2004). Evaluation of the Effect of Endodontic Irrigation Solutions on the Microhardness and the Roughness of Root Canal Dentin. Journal of Endodontics,Vol.30,No.11.p 792-794.

4. Duvvi SAB, Adarsha MS, Usha HL, Ashwini P, Murthy CS, Shivekshith AK.(2018)Comparative Assessment of Different Concentrations of Sodium Hypochlorite and Calcium Hypochlorite on Microhardness of Root Canal Dentin – An In Vitro Study. Int J Oral Care Res.

5. Oliveira LD, Carvalho CAT, Nunes W, Valera MC, Camargo CHR, Jorge AOC.(2007).Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin.Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007 and 104:e125-e128.

6.Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. Journal of dentistry. 1999 Jan 1 and 27(1):43-6.

7.Tsenova-Ilieva I, Karova E. The effect of different irrigants with or without ultrasonic activation on root dentin microhardness. Journal of IMAB–Annual Proceeding Scientific Papers. 2021 Jan 19 and 27(1):3534-8.

8.Ari H, Erdemir A, Belli S.(2004). Evaluation of the Effect of Endodontic Irrigation Solutions on the Microhardness and the Roughness of Root Canal Dentin. Journal of Endodontics,Vol.30,No.11.p 792-794.

9. Pashley D, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. Dental Traumatology. 1985 Oct and 1(5):176-9.

10. Pinto L, Cardoso I, Rover G, Bortoluzzi E, Garcia L, Teixeira CS. Effect of different irrigation protocols and calcium hydroxide dressing on the microhardness of root canal dentin. Giornale Italiano di Endodonzia. 2020 Oct 26 and 34(2).

11.Mohammadi Z, Jafarzadeh H, Shalavi S. Antimicrobial efficacy of chlorhexidine as a root canal irrigant: a literature review. Journal of Oral Science. 2014 and 56(2):99-103.

12. Athanassiadis B, Abbott PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. Australian dental journal. 2007 Mar and 52:S64-82.

13. Carlotto IB, Luisi SB, Kopper PM, Grecca FS, Montagner F. Calcium hypochlorite solutions: evaluation of surface tension and effect of different storage conditions and time periods over pH and available chlorine content. Journal of endodontics. 2016 Ap.

14. Patil CR, Uppin V. Effect of endodontic irrigating solutions on the microhardness and roughness of root canal dentin: an in vitro study. Indian Journal of Dental Research. 2011 Jan 1 and 22(1):22.

15. Samiei M, Farjami A, Dizaj SM, Lotfipour F.(2016).Nanoparticles for antimicrobial purposes in Endodontics : A systematic review of in vitro studies.Materials Science and Engineering C 58.1269-1278.

16.Pimenta JA, Zaparolli D, Pécora JD, Cruz-Filho AM. Chitosan: effect of a new chelating agent on the microhardness of root dentin. Brazilian dental journal. 2012 and 23(3):212-7.

17.Saha SG, Sharma V, Bharadwaj A, Shrivastava P, Saha MK, Dubey S, Kala S, Gupta S .(2017)Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentin: An in vitro Study. Journal of Clinical and Diagnostic Research. 2017.

18.Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. International endodontic journal. 2001 Mar and 34(2):120-32.

19. Grigoratos D, Knowles J, Ng YL, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. International endodontic journal. 2001 Mar and 34(2):113-9.

20. Doğan H, Çalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. Journal of endodontics. 2001 Sep 1 and 27(9):578-80.

21.Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. Journal of endodontics. 2004 Dec 1 and 30(12):880-2.

22. Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Sep and 104(3):418.

23.Cruz-Filho AM, Sousa-Neto MD,Savioli RN, Silva RG, Vansan LP, Pecora JD.(2011). Effect of Chelating Solutions on the Microhardness of Root Canal Lumen Dentin.JOE — Volume 37, Number 3, March 2011.358-362.

24.Silva PV, Guedes DF, Pécora JD, Cruz-Filho AM. Time-dependent effects of chitosan on dentin structures. Brazilian dental journal. 2012 and 23(4):357-61.

25.Dutta A, Saunders WP. Comparative evaluation of calcium hypochlorite and sodium hypochlorite on soft-tissue dissolution. Journal of Endodontics. 2012 Oct 1 and 38(10):1395-8.

26.Ulusoy Öİ, Görgül G. Effects of different irrigation solutions on root dentine microhardness, smear layer removal and erosion. Australian Endodontic Journal. 2013 Aug and 39(2):66-72.

27. Garcia AJ, Kuga MC, Palma-Dibb RG, Só MV, Matsumoto MA, Faria G, Keine KC. Effect of sodium hypochlorite under several formulations on root canal dentin microhardness. Journal of investigative and clinical dentistry. 2013 Nov and 4(4):229-32.

28.Das A, Kottoor J, Mathew J, Kumar S, George S. Dentine microhardness changes following conventional and alternate irrigation regimens: An in vitro study. Journal of conservative dentistry: JCD. 2014 Nov and 17(6):546.

29. Kandil HE, Labib AH, Alhadainy HA. Effect of different irrigant solutions on microhardness and smear layer removal of root canal dentin. Tanta Dental Journal. 2014 Apr 1 and 11(1):1-1.

30. Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, sodium hypochlorite, and chlorhexidine gluconate with or without surface modifiers on dentin microhardness. Journal of Endodontics. 2014 Jun 1 and 40(6):876-9.

31. Kalluru SR, Kumar ND, Ahmed S, Sathish ES, Jayaprakash T, Garlapati R, SowmyabB, Reddy KN.(2014) Comparative Evaluation of the Effect of EDTA,EDTAC, NaOCl and MTAD on Microhardness of Human Dentin – An In – vitro Study.Journal of Clinical and Diagnostic.

32.Oliveira JS, RaucciNeto W, Faria NS, Fernandes FS, Miranda CE, Rached-Junior FJ. Quantitative assessment of root canal roughness with calcium-based hypochlorite irrigants by 3D CLSM. Brazilian Dental Journal. 2014 Sep and 25:409-15.

33.Bakr DK, Saleem SS, Amin BK. Effect of sodium hypochlorite, chlorhexidin and EDTA on dentin microhardness. Zanco Journal of Medical Sciences (Zanco J Med Sci). 2016 and 20(1):1175_1179-.

34. Saleh HA. Comparative evaluation of effect of irrigation solutions with various exposure time on microhardness of root canal dentin (in vitro study). Iraqi Dental Journal. 2016 and 38(3):124-8.

35. Baldasso FE, Roleto L, Silva VD, Morgental RD, Kopper PM. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Brazilian oral research. 2017 May 15 and 31.

36. Massoud SF, Moussa SM, Hanafy SA, El Backly RM. Evaluation of the microhardness of root canal dentin after different irrigation protocols (in vitro study). Alexandria Dental Journal. 2017 Apr 1 and 42(1):73-9.

37. Quteifani M, Madarati AA, Layous K, Tayyan MA. A comparative ex-vivo study of effects of different irrigation protocols with/without laser activation on the root dentine's micro-hardness. European endodontic journal. 2019 and 4(3):127.

38. Unnikrishnan M, Mathai V, Sadasiva K, Santakumari RSM, Girish S, Shailajakumari AK. The Evaluation of Dentin Microhardness After Use of 17% EDTA, 17% EGTA, 10% Citric Acid, MTAD Used as Chelating Agents Combined With 2.5% Sodium Hypochlorite After Rot.

39. Dhawan R, Gupta A, Dhillon JS, Dhawan S, Sharma T, Batra D. Effect of different irrigating solutions with surfactants on the microhardness and smear layer removal of root canal dentin: An in vitro study. J Conserv Dent. 2019 Sep-Oct and doi, 22(5):454-458.

40. Barbosa AFS, Mello LM, Teixeira FG, Rached-Júnior FJA, Trindade TF, Raucci-Neto W. Comparative Effect of Rotary Microbrush Combined with Different Chemical Irrigants on Root Dentin Microhardness, Roughness and Bond Strength to an Epoxy-based Sealer. O.

41 Durigon M, Cecchin D, de Carli JP, Souza MA, Farina AP. Could calcium hypochlorite and grape seed extract keep the mechanical properties of root dentin and fracture resistance of weakened roots? J Mech Behav Biomed Mater. 2020 Jun and 10., 106:103736. doi:.

42..Bosaid F, Aksel H, Makowka S, Azim AA. Surface and structural changes in root dentine by various chelating solutions used in regenerative endodontics. Int Endod J. 2020 Oct and 32619296., 53(10):1438-1445. doi: 10.1111/iej.13354. Epub 2020 Jul 30. PMID:.

43. Sahebi S, Sobhnamayan F, Moazami F, Naseri M. Assessment of sodium thiosulfate neutralizing effect on micro-hardness of dentin treated with sodium hypochlorite. BMC Oral Health. 2020 Nov 12, 33183300, 20(1):326. doi: 10.1186/s12903-020-01320-2. PMID: and P.

44. Kour S, Malik A, Choudhary A. Effect of different irrigation porotocols on root dentine micro-hardness-An invitro study. IP Indian Journal of Conservative and Endodontics. 2021 Dec 15 and 6(4):217-21.

45. Elika V, Kunam D, Anumula L, Chinni SK, Govula K. Comparative evaluation of Chloroquick with Triphala, sodium hypochlorite, and ethylenediaminetetraacetic acid on the microhardness of root canal dentin: An in vitro study. J Clin Transl Res. 2021 Jan 2.

46. Arul B, Suresh N, Sivarajan R, Natanasabapathy V. Influence of volume of endodontic irrigants used in different irrigation techniques on root canal dentin microhardness. Indian J Dent Res. 2021 Apr-Jun and PMI, 32(2):230-235. doi: 10.4103/ijdr.IJDR_709_18.

47.Philip PM, Sindhu J, Poornima M, Naveen DN, Nirupama DN, Nainan MT. Effects of conventional and herbal irrigants on microhardness and flexural strength of root canal dentin: An in vitro study. J Conserv Dent. 2021 Jan-Feb and 10.4103/JCD, 24(1):83-87. doi:.

48. Kulkarni S, Mustafa M, Ghatole K, AlQahtani AR, I Asiri FY, Alghomlas ZI, Alothman TA, Alhajri FF. Evaluation of 2% Chlorhexidine and 2% Sodium Fluoride as Endodontic Irrigating Solutions on Root Dentine Microhardness: An In Vitro Study. Eur J Dent. 2.

49.Tsenova-Ilieva I, Karova E. The effect of different irrigants with or without ultrasonic activation on root dentin microhardness. Journal of IMAB–Annual Proceeding Scientific Papers. 2021 Jan 19 and 27(1):3534-8.

50.Kaiwar A, Usha H L, Meena N, Ashwini P, Murthy CS. The efficiency of root canal disinfection using a diode laser: In vitro study. Indian J Dent Res 2013 and 24:14-8.

51.Ashraf F, Shankarappa P, Misra A, Sawhney A, Sridevi N, Singh A. A stereomicroscopic evaluation of dentinal cracks at different instrumentation lengths by using different rotary files (ProTaper Universal, ProTaper Next, and HyFlex CM): An Ex Vivo Study.

52.Tortini D, Colombo M, Gagliani M. Apical crown technique to model canal roots. A review of the literature. Minerva Stomatologica. 2007 Sep 1 and 56(9):445-59.

53.Baugh D, Wallace J. The role of apical instrumentation in root canal treatment: a review of the literature. Journal of endodontics. 2005 May 1 and 31(5):333-40.

54.Topçuoğlu HS, Düzgün S, Akpek F, Topçuoğlu G. Effect of glide path and apical preparation size on the incidence of apical crack during the canal preparation using Reciproc, WaveOne, and ProTaper Next systems in curved root canals: A stereomicroscope st.

55.Brunson M, Heilborn C, Johnson DJ, Cohenca N. Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. Journal of endodontics. 2010 Apr 1 and 36(4):721-4.

ANNEXURES

ANNEXURE I

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIII[th] Institutional Ethics Sub-Committee

IEC Code: 10 **BBDCODS/03/2020**

Title of the Project: A Comparative Evaluation of the Effect of Different Endodontic Irrigating Solutions on Microhardness of Root Canal Dentin: *An In-Vitro Study.*

Principal Investigator: Dr. Chris Cherian Geogi **Department:** Conservative Dentistry & Endodontics

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: New, MDS Project Protocol

Dear Dr. Chris Cherian Geogi,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 18[th] March, 2020.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sahana S. Member	Reader, Department of Public Health Dentistry, BBDCODS, Lucknow
4.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.

The comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala
18/03/20

(Dr. Lakshmi Bala)
Member Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Sciences

ANNEXURE II

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"A Comparative Evaluation of the Effect of Different Endodontic Irrigating Solutions on Microhardness of Root Canal Dentin: *An In-Vitro Study*"** submitted by **Dr Chris Cherian Geogi** Post graduate student from the **Department of Conservative Dentistry and Endodontics** as part of MDS Curriculum for the academic year 2019-2022 with the accompanying proforma was reviewed by the Institutional Research Committee present on **19th December 2019** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

ANNEXURE III

Curiginal

Document Information

Analyzed document	cherian plagiarism.docx (D132407290)
Submitted	2022-04-03T07:17:00.0000000
Submitted by	
Submitter email	1180328003@bbdu.ac.in
Similarity	8%
Analysis address	1180328003.bbduni@analysis.urkund.com

Sources included in the report

W	URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5754985/ Fetched: 2019-10-24T09:32:30.9230000	1
W	URL: http://www.ijocrweb.com/pdf/2018/January-March-Supplementary/12_DR%20ADARSH_OA.pdf Fetched: 2020-12-25T05:52:15.5200000	8
W	URL: https://www.ijsr.net/archive/v9i4/SR20406125440.pdf Fetched: 2022-04-02T11:39:58.6770000	2
W	URL: https://www.researchgate.net/publication/337346574_EVALUATION_OF_THE_MICROHARDNESS_OF_ROOT_CANAL_DENTIN_AFTER_DIFFERENT_IRRIGATION_PROTOCOLS_IN_VITRO_STUDY Fetched: 2022-04-03T07:17:30.3700000	1
W	URL: https://edj.journals.ekb.eg/article_75261_61199f05bb102060c0568d5f8b9ec065.pdf Fetched: 2022-04-03T07:19:22.5870000	8
W	URL: https://www.researchgate.net/publication/308647748_Effect_of_5_Calcium_Hypochlorite_on_Mechanical_Properties_of_Root_Dentin_An_in_vitro_Study_1 Fetched: 2019-12-03T09:25:59.4370000	1
W	URL: https://biomedpharmajournal.org/vol11no4/effect-of-final-irrigation-protocol-on-dentin-microhardness-2/ Fetched: 2020-02-17T12:30:01.0370000	1

ANNEXURE IV

PRAJ
Metallurgical Laboratory

B-38, Indira Shankar Nagari,
Paud Road, Near Kothrud PMT Depo,
Kothrud, Pune - 411 038
Tel.: +91 - 20 - 2528 1584,
Cell : 96650 11314, 9158658634
E-mail : prajdental@gmail.com
Website: www.prajlab.com

DENTAL MATERIAL TEST FACILITY
MDS Thesis in various fields
i.e. Orthodontic, Prosthodontics, Endodontics, Periodontics, Oral Surgery, etc.

Date: 27/01/2022

TO WHOMSOEVER IT MAY CONCERN

This is to cerify that Dr. Chris Cherian Geogi, final year MDS, Babu Banarasi Das College of Dental Sciences, has done his Vickers Microhardness Testing of Teeth Samples from PRAJ Metallurical Laboratory, Kothrud, Pune.

TEST CONDUCTED BY

SACHIN
TEST ENGINEER

A.M.BHAGAT
PROPRIETOR

PRAJ METALLURGICAL LABORATORY ★ PUNE