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Evaluation of chemical composition of root canal dentin between two age groups using different irrigating solutions: An in vitro sem-eds study

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ABSTRACT

Objectives: The study aimed to evaluate and compare the effect of different irrigating solutions on the chemical composition of root canal dentin between two age groups.
Materials a nd Methods: Sixty-six extracted single rooted human premolar teeth were decoronated and longitudinally sectioned to expose canals. Root halves were divided into 2 groups and 3 sub-groups based on the irrigation protocol. Samples were evaluated for mineral content with Scanning Electron Microscope/Energy Dispersive Spectroscopy (SEM/EDS). The data were analyzed statistically.
Results: Results revealed a statistically significant difference in calcium levels of younger age group, with higher levels for SmearClear (19.1±1.31) when compared to MTAD (Mixture of Tetracycline Isomer, Acid and Detergent) (17.1±1.16), with p value 0.01. A statistically significant difference was observed in magnesium levels of younger age group, with higher levels for MTAD (0.95±0.21) when compared to SmearClear (0.82±0.22), with p value 0.05. Mean comparison of Ca/P ratio of younger age group, revealed higher values for SmearClear (1.71±0.14) when compared to MTAD (1.56±0.15), with p value 0.02.
Conclusion: MTAD caused significantly greater loss of calcium, phosphorous, sodium and altered Ca/P ratio of root dentin among younger age group. On the other hand, loss of magnesium content was found to be higher within SmearClear sub group of younger age group (p=0.05).

1. Introduction

The key to successful endodontic therapy is to ensure complete eradication of microbial volume, which can be accomplished by cleaning and shaping phase of root canal therapy.[1] leads to accumulation of organic pulpal material and inorganic dentinal debris producing an amorphous, irregular surface layer known as "Smear layer".[2] bacterial harbouring potential of smear layer within dentinal tubules prevents proper disinfection as well as penetrability of sealer and adhesion of obturation materials.[3]

Irrigation is considered to be a vital adjunct to instrumentation of root canals. Hence, it has been agreed that "files shape and irrigants clean".[4] Sodium hypochlorite (NaOCl), commonly used irrigation agent has a potential to dissolve organic matter and necrotic tissue, also prevents greater dentin dissolution, but leaves a smear layer of mineralized tissue.[5] MTAD (Mixture of Tetracycline Isomer, Acid and Detergent) a synergistic combination of 3% doxycycline, 4.25% citric acid and detergent (Tween-80) introduced by Torabinejad had significantly improved smear layer removal as it is considered to be a chelating agent and also induces inconsiderable erosion. A new formulation of EDTA (Ethylene Diamine Tetraacetic Acid), SmearClear containing 17% EDTA, Cetrimide and a

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specific surfactant have gained increasing attention in recent years. [6]

Dentin composition is described based on its organic and inorganic constituents. Calcium and phosphorus present in hydroxyapatite crystals are considered to be the major inorganic components of dental hard tissues. Any change in the Ca/P ion ratio may alter the standard proportion of the organic and inorganic components in the dentin which in turn affects the micro-hardness, permeability as well as solubility characteristics of dentin, sealing ability and adhesion of dental material to the root canal dentin [7] while performing endodontic therapy are considered to be critical for the success of the treatment. The continuous deposition of peritubular dentin, consequent narrowing of dentinal tubules, and a decrease in the number of dentinal tubules are commonly seen among older individuals as these changes tend to correlate with the natural ageing process of humans. [8]

Various studies interpolating the above factors have been primarily focused only on efficiency of on the chemical composition of dentin. Hence, the present study aimed to evaluate and compare the effect of different irrigating solutions on the chemical composition of root canal dentin between two age groups.

2. Materials and Methods

2.1. Preparation of specimens

Sixty-six single rooted human premolar teeth extracted from the subjects aged 18-25 years and 47-65 years, (33 from each group) for orthodontic and periodontal reasons were collected from the Department of Oral and Maxillofacial Surgery, S.V.S. Institute of Dental Sciences, Mahabubnagar. Teeth with open apex, anatomical variations and sharp curvatures were from the study. Surfaces of collected human premolar teeth were visually examined with naked eye for any macroscopic surface defects, cleaned of debris and calculus using an ultrasonic device and stored in distilled water till the experiment was initiated. Specimens from each group were then divided randomly into three subgroups A, B and C (n= 11) based on the irrigant used as follows:

	Group-1 Young Group (18-25 years)	**Group-2 Old Group (47-65 years)**
SUB Group A	NaOCl	NaOCl
SUB Group B	MTAD	MTAD
SUB Group C	SmearClear	SmearClear

Experiment Patency of the canal was checked with #10 K-file (fig-9) and canals were instrumented till #15 K-File and subsequently with ProTaper Ni-Ti rotary instrument sequence until master apical file #F3. Between each file, canalirrigated with 2 mL 3% NaOClfinal irrigation is done with:

	Group-1 Young Group (18-25 years)	**Group-2 Old Group (47-65 years)**
SUB Group A	1ml of 0.9% Saline for 1 minute	1ml of 0.9% Saline for 1 minute
SUB Group B	1ml of MTAD for 1 minute	1ml of MTAD for 1 minute
SUB Group C	1 ml of SmearClear for 1 minute	1 ml of SmearClear for 1 minute

All samples were then dried with sterile absorbent paper points and grooved longitudinally in a buccolingual plane with a slow speed diamond disc and split into two halves using a chisel and mallet and stored in distilled water until use. The samples from each group are subjected to energy dispersive spectroscopy to determine the alteration in Ca, P, Na and Mg caused by the various irrigants

2.2. Post experimental evaluation of mineral content of specimens

The specimens were secured on metallic (aluminium) stubs, dried in the critical point dryer, sputter coated with gold and analysed under (JOEL/JSM-IT500A) at 20 kV, in which the specimens are bombarded with a high-voltage electron beam that generates different wavelengths for each type of mineral. The change in wavelength of the radiation that is emitted by the specimens is used to determine the post-experimental atomic percentage of Ca, P, Na, Mg.

2.3. Statistical analysis

Data was entered and analyzed using SPSS (Statistical Package for Social Sciences) software 26.0 (IBM, New York). Descriptive statistics were presented as means and standard deviations for numerical variables, or as frequencies and percentages for categorical variables. Inferential statistics included independent sample t-test for comparison of means between two different age groups. The level of significance was set at $p \leq 0.05$.

Results revealed a statistically significant difference in calcium levels of younger age group, with higher levels for SmearClear (19.1±1.31) when compared to MTAD (17.1±1.16), with p value 0.01(Table 1). A statistically significant difference was observed in magnesium levels of younger age group, with higher levels for MTAD (0.95±0.21) when compared to SmearClear (0.82±0.22), with p value 0.05 (Figure 1). Mean comparison of Ca/P ratio of younger age group, revealed higher values for SmearClear (1.71±0.14) when compared to MTAD (1.56±0.15), with p value 0.02 (Table 2).

Independent sample t-test revealed statistically significant difference in phosphorous levels of older

age group, with higher levels for SmearClear (13.9±1.90) when compared to MTAD (12.7±1.32), with p value 0.03 (Table-3). Statistically significant difference was observed in magnesium levels and Ca/P ratio of older age group, with higher levels for SmearClear (1.27±0.26) & (1.49±0.30) when compared to MTAD (1.21±0.45) & (1.42±0.24), with p value 0.05 (Figure 2).

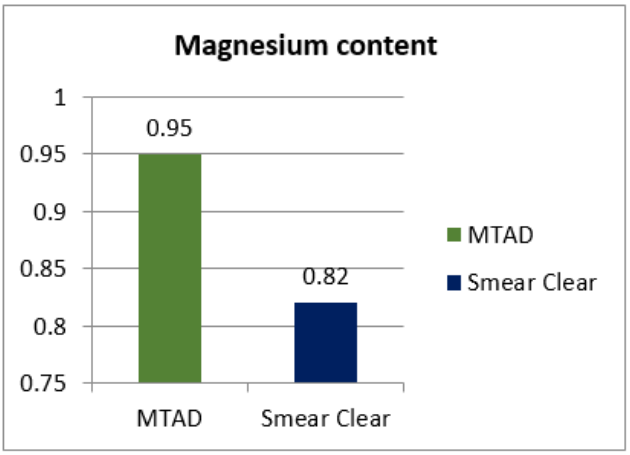

Figure 1: Mean comparison of magnesium content between irrigants within young group

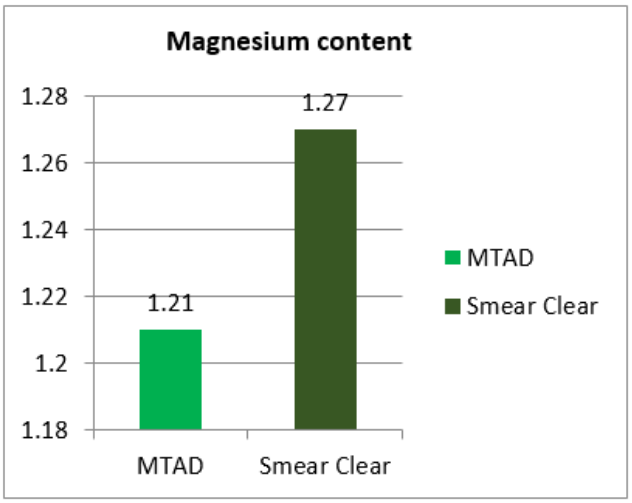

Figure 2: Mean comparison of magnesium content between irrigants within old group

3. Discussion

Successful root canal treatment is focused on the complete removal of micro-organisms and their products by meticulous chemo-mechanical preparation followed by three-dimensional obturation of the root canal system.[9] An inevitable outcome of any hand or rotary instrumentation is the creation of a substantial amount of debris shattered from

Table 1: Mean comparison of calcium content between irrigants within young group

Groups	Calcium content Group 1 (Younger age group) Mean	SD
MTAD	17.1364	1.16975
Smear Clear	19.1964	1.31468
p value	0.01*	

Table 2: Mean comparison of Ca/P ratio between irrigants within young group

Groups	Ca/P Ratio Group 1 (Younger age group) Mean	SD
MTAD	1.5682	0.15452
Smear Clear	1.7164	0.14445
p value	0.02*	

Table 3: Mean comparison of phosphorous content between irrigants within old group

Groups	Phosphorous content Group 2 (Older age group) Mean	SD
MTAD	12.7264	1.32706
Smear Clear	13.9127	1.90919
p value	0.03*	

mineralized tissues forming a non-homogenous structure, called the smear layer, on the walls of the cavity and the root canal. Clinically produced smear layers have an average depth of 1-5 microns (Goldman et.al.1981, Mader et.al. 1984).

Root canal therapy should not be simply the extirpation of the pulp and widening of the canal, but one should also focus on how to completely remove the loosely-attached smear layer, through irrigation of root canal space. However, irrigants used in the removal of smear layer produced during root canal preparation, might also affect the microhardness, chemical composition of root dentin.[10] Any change in the mineral content such as Ca/P ratio may alter the standard proportion of the organic and inorganic components in the dentin which in turn affects the microhardness, permeability, solubility characteristics of dentin, sealing ability and adhesion of dental materials to the root canal dentin. Specifically, in case of root dentin, the irrigating agents react with the calcium ions in the hydroxyapatite crystals and can cause changes in the microstructure of the dentin by changing its Ca/P ratio.[11]

The age-related changes of the dentin are also considered to be one of the crucial factors for the success of the endodontic treatment. The continuous deposition of peritubular dentin, consequent narrowing of dentinal tubules and decrease in the number of dentinal tubules are commonly seen among older individuals as these changes

tend to correlate with the natural ageing process of humans.[7]

Evidence shows that endodontic instruments are unable to contact all the parts of the root canal wall, leaving behind untouched areas. Thus, the use of chemical adjuncts is considered a "sine qua non" in root canal treatment.

Sodium hypochlorite the most commonly used irrigant, has the ability to dissolve the organic tissues. It has been reported that the use of NaOCl alone also altered the mineral content of root dentin causing mineral accumulation and increased amount of carbonate and reduced amount of phosphate.[12]

The most common chelating solutions are based on Ethylene diaminetetraacetic acid (EDTA). EDTA reacts with the calcium ions present in root dentin and forms soluble calcium chelates and causes demineralization of dentin. The disadvantage is that it has negligible smear layer removal efficacy in the apical third which is a critical area for disinfection and obturation.[13]

Later, MTAD (Mixture of Tetracycline Isomer, Acid and Detergent) a synergistic combination of 3% doxycycline, 4.25% citric acid, 0.5% polysorbate and detergent (Tween-80) introduced by Mahmoud Torabinejad had significantly improved smear layer removal as it is considered to be a chelating agent and also induces inconsiderable erosion.[14] In a recent investigation, De-Deus et al., reported significantly faster dissolution of inorganic material by both 5% citric acid and MTAD as compared with 17% EDTA.[15]

A new formulation of EDTA, SmearClear containing 17% EDTA, cationic surfactant Cetrimide and a specific anionic surfactant Triton X-100 has gained increasing attention in recent years due to significant removal of smear layer in the apical third of root canal when compared to EDTA alone.[16]

Surface alteration in root dentin may be due to the chemical changes brought by the irrigants resulting in alteration of Ca/P ratio[17] and these changes can be observed under Scanning Electron Microscope (SEM) whereas Energy Dispersive Spectroscopy (EDS) can be used for elemental analysis of tooth material to detect changes in Ca, P, Na, Mg and Ca/P ratio of root dentin.[18]

In this study SEM/EDS is used which is based on the energy emitted in the form of X-ray photons, when the electrons from external source hit the atoms in a material, they generate characteristic X-rays of that element. This method allows fast and quantitative microanalysis, estimating the amount of mineral in a given tooth sample in a non-destructive manner.[19]

In the present study, comparison of irrigants within younger age group revealed statistically significant difference in calcium content and Ca/P ratio with higher values for SmearClear sub group. This may be due to the presence of citric acid in MTAD which is a strong chelating agent and hence removed more calcium ions from root canal dentin.[20]

Also, a statistically significant difference was observed in magnesium content between SmearClear and MTAD with higher values for MTAD sub group. This could be due to the binding of magnesium to phosphate, carbonate or the organic matrix.[21] This finding is in contrary with the study conducted by Eren SK et al., where the magnesium level changes were not significant when chelators are used as a final irrigant.[19]

A statistically significant difference was observed in phosphorous content with higher values for SmearClear sub group. This is in accordance with Tay and Pasley who stated that mechanically instrumented intraradicular dentin after irrigation with EDTA or MTAD created a zone of demineralized collagen matrices in eroded dentin causing mineral loss with the mildly acidic MTAD being more aggressive than EDTA.[22]

The organic matrix of dentin may act as a limiting factor in the dissolution of the inorganic component, thus reducing the decalcifying action of chelating agents over time. However, the low pH of irrigating solutions facilitates increased removal of major inorganic elements like calcium from the hydroxyapatite crystals.[23] Probably, this could be one of the reason for greater loss of minerals from MTAD (pH = 2.15) when compared to SmearClear (pH = 7.5-9).

Also, the current study is in line with the study conducted by Weerakoon AT et al, where the mineral contents were found to be higher among younger dentin samples. It could be ascribed to physiological ageing, where ageing increases dentin apatite density and distribution but not composition.[24]

However, the present study acknowledges certain limitations. Firstly, the in vitro design of study is the major limitation along with the limited sample size. Blood, tissue remnants, temperature, various delivery and agitation devices may affect the action of chelating agents used during the root canal instrumentation.

Also, mineral content of dentin vary depending on the anatomical location, therefore separate measurements from each root third are to be considered and not of single point. Other factors like mineral content of the samples before treatment were not considered in the study due to logistic reasons. Future studies should include comparison of baseline with post-experimental mineral content of root dentin.

4. Conclusion

Within the limitations of this in vitro investigation, the following conclusions were drawn:

1. MTAD caused significantly greater loss of calcium, phosphorous, sodium and altered Ca/P ratio of root dentin among younger age group.

2. On the other hand, loss of magnesium content was found to be higher within SmearClear sub group of younger age group (p=0.05).
3. Similarly, among older age group, a significantly greater loss of all the minerals (calcium, phosphorous, magnesium and Ca/P ratio) were noted in MTAD sub group except for sodium content, wherein predominantly higher loss of sodium was observed in SmearClear sub group ($p \leq 0.05$)

5. Conflict of Interest

No conflict of interest.

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