Comparative Evaluation of Calcium Ion Loss and Microhardness using Different Irrigants - An In Vitro Study

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ABSTRACT

Objective: Evaluation along with comparison of 18% Hydroxyethylidene bisphosphonate (HEBP), 15% Citric acid and 5.25% Sodium hypochloride on calcium loss and resultant changes in microhardness of the root dentin. **Material And Method:** Twenty single rooted mandibular premolars were decoronated, and a transverse section of 2 mm was prepared from the coronal third of the root. These sections were divided into 3 groups. Group 1 (Control) was treated with 5.25% Sodium hypochlorite (NaOCl) + distilled water, group 2 with 5.25% NaOCl + 18% HEBP and group 3 with 5.25% NaOCl + 15% Citric acid. The calcium loss was evaluated using the Atomic Absorption Spectrophotometer and microhardness with Vickers Hardness Tester. Data was statistically evaluated using one–way ANOVA, Post hoc Tukey test along with Pearson correlation. **Results:** Calcium loss along with resultant drop in microhardness were maximal in Group 3 followed by Group 2 and Group 1. Statistically significant difference was observed between the groups other than between Group 2 and 3. **Conclusions:** The use of 5.25% NaOCL, 18 % HEBP or 15% Citric acid results in calcium loss which alter the microhardness of root dentin, mainly during the first 5 min of action.5.25% sodium hypochlorite solution is capable of extracting small amounts of calcium from root dentin as compared to HEBP, and maximum loss occurs with Citric acid.

KEYWORDS: Citric Acid, Hydroxyethylidene bisphosphonate (HEBP), Sodium hyphochlorite, Root microhardness

INTRODUCTION

Root canal treatment is a series of procedures which include disinfection and sealing of the root canal system. Dentin surface gets covered with smear layer while shaping and cleaning using various endodontic instruments. Smear layer reduces dentinal permeability which hinders the penetration of chemicals and intracanal medicaments. It also effects the penetration of sealer in the lateral canal and adherence of epoxy/resins gutta percha to the root canal as a final filling. This prevents adequate cleaning and disinfection of the root canals.[1]

Chelating agents can be used along with sodium hypochlorite for removal of smear layer. Their effect on dentine is important and can cause an alteration in chemical structure by the loss of Ca and K ions resulting in a change of physical and mechanical properties of dentine; deprotienation of root surface finally causing collagen loss. Most researchers have analyzed only the ability to chelate solutions to remove the smear layer .[2]

In the recent past materials like EDTA, Peraceticacid and HEBP have been introduced which can be used along with other irrigants like NaOCl, for removal of smear layer. Studies have reported that EDTA or citric acid strongly reacts with NaOCl, thus making the latter ineffective.[3] Consequently, etidronic acid (1‐hydroxy-ethylidene‐1,1 bisphosphonate or HEBP) has been investigated as a potential alternative. HEBP is nontoxic and has been systematically used to treat bone diseases.[4] However correlation of Ca ion loss and its effect on the strength of root dentine arising with use of HEBP has not been studied extensively.

Thus ,this study was taken up to evaluate the effect of HEBP when used as chelating agent on the calcium loss and its effects on the strength and micro hardness of the root dentin.

MATERIALS AND METHODS

Twenty freshly extracted single rooted intact human premolars were collected from the OPD of the department of Oral and Maxillofacial surgery; RKDF dental college and research centre, Bhopal, India .Teeth were cleaned, free of debris and calculus using the ultrasonic scaler. They were stored for one week in formalin and later in normal saline until used.

Teeth extracted for orthodontic purpose or periodontal reasons, with complete root formation, patent canals and

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without anatomic variations were selected after verification with digital radiography. Teeth with caries or restoration, internal resorption, fracture or micro cracks, decalcification, incomplete root formation and with flourosis were excluded.

Decoronation of teeth was carried out at the cementoenamel junction with a diamond disk at high speed. 2 mm thick transverse sections were obtained from the coronal third of each root with the help of slow speed diamond disc. Each section was further divided into 4 halves, resulting in 4 sections. Out of these 3 sections were taken up for the study. To the exposed canal part of the dentin, specimens were horizontally embedded in autopolymerizing resin. A total of 60 samples were prepared and equally divided into three groups –Group I, II and III.

Preparation of irrigants: Irrigants were freshly prepared by mixing distilled water to the HEBP powder (Zschimmer and Schwarz Mohsdorf GmbH and Co., KG, Burgstadt, Germany). Thus, a solution of w/v concentrations of 18% was prepared. 5.25% of commercially available NaOCl and 15% citric acid solution was used.

In Group I, samples were treated with 5.25% NaOCl and distilled water for 5 minutes each, whereas in Group II, with 5.25% NaOCl for 5 minutes & 18% Etidronate for 5 minutes and in Group III, with 5.23 % NaOCL for 5 min & 15% Citric Acid for 5min.

Evaluation of Calcium loss: Samples were placed in the freshly prepared solutions within the magnetic stirrer bath. The samples were placed in 10 ml of hypochlorite for 5 min followed by rinsing and placement in the other solution for 5 minutes. After irrigation of each specimen in both groups, the eluates were centrifuged at 4000 g for 10 min. 20ml of total eluate per specimen was collected in individual glass vials. Subsequently, 10 ml of the supernatant was transferred to a tube with a lid and stored for further analysis.

After collection of all the eluates, they were analyzed for calcium content using atomic absorption spectrophotometer with an air acetylene flame. Measurements were performed, and the results were expressed as ppm Ca^{2++} in eluates.

Microhardness measurement: After the combined treatment of each specimen, surface hardness for the root dentine was checked with Vickers hardness tester. A load of 300g for 15 seconds was used for measuring the hardness. For each sample,three indentations were made, and hardness value was obtained by calculating the average of three indentation values.

Statistical analysis: For each group mean and standard deviation were estimated. Mean values obtained from the different groups were comparatively evaluated with one – way ANOVA and than *post hoc* Tukey test. For establishing the relationship between calcium loss and subsequent microhardness Pearson's correlation test was utilized.

RESULTS

Average calcium loss for the group 1 was found to be 2.7925 ppm whereas average microhardness was 77.29 VHN(ranging from 76.09 to 77.98) (Table1). Average calcium loss for group 2 was found to be 6.374 ppm whereas average microhardness was 69.053 VHN(ranging from 68.56 to 69.77)(Table 2). Average calcium loss for group 3 was found to be 6.796 ppm whereas average microhardness was 66.804 VHN(ranging from 59.03 to 68.98)(Table 3). For both calcium loss and microhardness, a statistically significant difference was found between the three groups. The negative correlation was found between calcium loss and reduction in microhardness of root dentine in both the groups (table 4). Distribution and comparison of calcium loss and microhardness in various groups can be appreciated in graph 1 & 2.

GROUP 1 5.25%NAOCL+Distilled Water		Calcium Loss (ppm)	Microhardness (Vhn)
1	1	2.32	77.11
2	1	2.41	77.03
3	1	2.53	77.08
4	1	2.59	77.98
5	1	2.72	77.96
6	1	2.32	77.67
7	1	2.83	76.09
8	1	2.89	77.41
9	1	3.15	77.99
10	1	2.59	76.96
11	1	3.18	78.12
12	1	3.21	77.41
13	1	2.56	76.06
14	1	2.78	77.95
15	1	2.42	77.87
16	1	3.24	76.89
17	1	2.94	76.98
18	1	2.46	77.07
19	1	3.19	77.12
20	1	3.52	77.15
	Mean	**2.7925**	**77.295**

Table 1. Calcium Loss And Microhardness Value In 5.25% Naocl And Distilled Water

Group 2 5.25% NaOCl+18% HEBP		CALCIUM LOSS (ppm)	MICROHARDNESS (VHN)
1	2	5.78	69.71
2	2	5.82	68.89
3	2	5.92	68.78
4	2	7.01	69.77
5	2	6.14	68.65
6	2	7.03	68.75
7	2	6.13	68.95
8	2	5.87	68.67
9	2	6.45	68.85
10	2	6.87	68.67
11	2	6.16	69.67
12	2	5.84	68.85
13	2	5.98	69.79
14	2	6.14	68.67
15	2	6.23	68.56
16	2	7.81	68.59
17	2	6.52	69.74
18	2	6.51	69.44
19	2	7.06	69.32
20	2	6.21	68.74
	Mean	**6.374**	**69.053**

Table 2. Calcium loss and microhardness value in 5.25% NaOCl and 18% HEBP

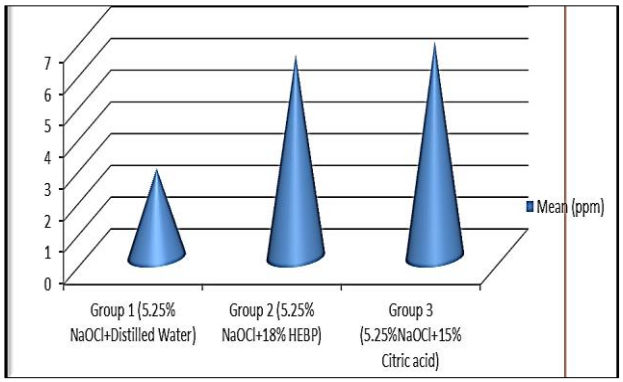
	Group 2 5.25%NaOCl+15% HEBP	Calcium Loss (ppm)	Microhardness (Vhn)
1	3	6.56	66.54
2	3	5.19	67.23
3	3	6.03	68.55
4	3	6.98	67.46
5	3	4.87	68.02
6	3	6.18	67.36
7	3	5.36	67.02
8	3	7.57	66.05
9	3	7.46	67.21
10	3	6.89	67.43
11	3	7.46	68.07
12	3	7.14	66.98
13	3	7.89	66.54
14	3	6.77	68.66
15	3	6.98	68.97
16	3	6.53	68.98
17	3	7.68	59.87
18	3	6.67	68.56
19	3	7.65	67.56
20	3	8.06	59.03
	Mean	**6.796**	**66.8045**

Table 3. Calcium loss and microhardness value in 5.25% NaOCl and distilled water

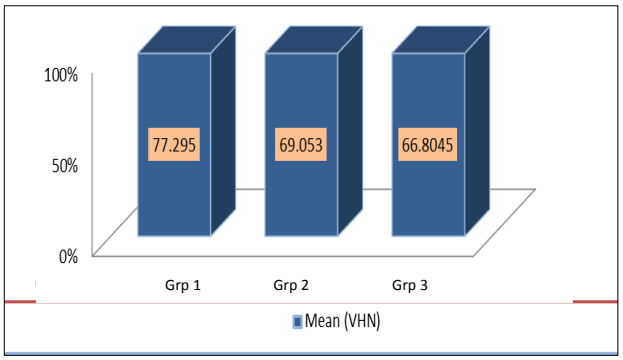
Pearson`s Correlations							
Groups and Variables		**Group 1 Calcium**	**Group 1 Micro hardness**	**Group 2 Calcium**	**Group 2 Micro hardness**	**Group 3 Calcium**	**Group 3 Micro hardness**
Group 1 Calcium	Pearson Correlation	1	.067	.185	-.119	.482*	-.401
	N	20	20	20	20	20	20
Group 1 Micro hardness	Pearson Correlation	.067	1	.043	-.179	.034	.219
	N	20	20	20	20	20	20
Group 2 Calcium	Pearson Correlation	.185	.043	1	-.052	.101	.127
	N	20	20	20	20	20	20
Group 2 Micro hardness	Pearson Correlation	-.119	-.179	-.052	1	.340	-.201
	N	20	20	20	20	20	20
Group 3 Calcium	Pearson Correlation	.482*	.034	.101	.340	1	-.446*
	N	20	20	20	20	20	20
Group 3 Micro hardness	Pearson Correlation	-.401	.219	.127	-.201	-.446*	1
	N	20	20	20	20	20	20

*Correlation is significant at the 0.05 level.

Table 4. Correaltion Between Calcium Loss & Microhardness



Graph 1: Distribution and comparison of Mean Calcium loss in all groups



Graph 2: Distribution and comparison of Microhardness

DISCUSSION

Dentin is composed of the various inorganic component of dental hard tissue, present as hydroxyapatite crystals in the form of calcium and phosphorous. In hydroxyapatite crystals Ca/P ratio has been established at 1.67 approximately. This ratio depends on many factors such as level of mineraliation, type of crystals, the age of tissue and anatomical site.[5]

During biomechanical preparation removal of smear layer requires the use of irrigant which can remove both organic and inorganic components of smear layer. The NaOCl irrigation when used alone acts only on the organic component which alters its physical and mechanical properties. If NaOCl is used consequently with chelating agents, it is capable of dissolving organic remnants and predentin or even demineralizing the inorganic calcified portion of the root canal wall[6].EDTA is the most widely used chelating agent ,but it tends to reduce the root strength.Citric acid is another widely accepted irrigant, displaying biocompatibility compared to other chelating agents,action on anaerobic microorganism and effectivesmear layer removal.[7] Citric acid also tends to reduce strength of the root, at the same time it has disaggreable taste and odour.[8]

According to Lottani et al. 2009, etidronic acid/sodium hypochlorite mixture could be administered as the sole irrigant. A combination of HEBP and NaOCl employed as an irrigant during and later after the biomechanical preparation ,does not lead to short term loss of the desires properties.[7]

Atomic absorption spectroscopy,was used in this study for determining the concentration of calcium thus evaluating the demineralizing effect of chelators. It is a single element technique, easy to use with high precision values and is cost-effective than newer multi-element techniques, like inductively coupled plasma atomic emission spectrometry.[9]

Alteration in Ca/P ratio of dentin results in changes in microhardness.[7] Vickers microhardness test was done to check the dental hard tissues surface changes resulting from the use of chelating agents. Over the other hardness

tests,the shape of the Vickers indentation is geometrically similar at all test loads. The hardness value is constant, within statistical precision and test load range, with the test specimen remaining homogeneous.

In the present study, teeth were decoronated from CEJ and four sections were obtained from the same tooth to ensure similarity in size and shape. This makes the chelators act homogeneously for calcium ion removal from the root dentin.

Hydroxyethylidene bisphosphonate also known as Etidronate, that prevents bone resorption and is used in patients suffering from osteoporosis as Paget's disease.[4,10] Thus, both the agents showing almost same result can be used as a chelating agents but when compared citric acid is still not preferred as it precipitates in the canal to form crystals, harbouring the microorganism and effecting the three dimensional seal of the root canal.[11]To evaluate that the weak chelators does not affect the microhardness and cause less calcium loss, so that they can be used in future in place of strong chelators which make root dentin weak by more calcium loss.[12] The main concern while using a new compound clinically is the question of its potential side effects. Etidronic acid is biocompatible and is used as an additive in various personal care products such as soaps (Licata 1993).[13] It is also used in swimming pools because of its compatibility with hypochlorite to prevent stains from metal ions. However, etidronic acid is a bisphosphonate, and the systemic administration of bisphosphonates has been linked to osteonecroses of the jaws (Migliorati et al. 2005). [14] On the other hand, no such reports have appeared related to etidronate, only to other bisphosphonates with a much higher capacity to inhibit osteoclast function (Krueger et al. 2007).[15] Zehnder et al. found that a 10% citric acid solution significantly removes more calcium than a 15.5% EDTA solution.[16] Spano et al. evaluated the concentration of calcium ions by using root canal chelators according to flame atomic absorption spectrometry and suggested that a solution of 15% EDTA is better at removing calcium ions than a solution of 10% citric acid.[17,18]

CONCLUSION

The use of solutions of 5.25% NaOCL,18 % HEBP or 15% citric acid results in calcium loss which alters the microhardness of root dentin, mainly during the first 5 min of action. It was also observed that 5.25% sodium hypochlorite solution is capable of extracting small amounts of calcium from root dentin as compared to HEBP and maximum loss with Citric acid

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