

Effect of the Various Combinations of Intra-coronal Bleaching Agents on Dentin Micro-hardness: An In Vitro Study

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ABSTRACT

Introduction: Intra-coronal bleaching, though is an established, simple, cost-effective and conservative method of improving the shade of root canal treated discolored anterior teeth, have shown some effects on microhardness of enamel and dentin, biomechanical properties of dental hard tissues and also the ultrastructure and mineral content of dentin. This research is conducted to evaluate the change in dentin micro-hardness using intra-coronal bleaching agents in combinations.

Objective: The aim of this study was to evaluate and compare the microhardness of human dentin following the use of intra-coronal bleaching agents in various combinations.

Materials and Method: A total of 52 samples of extracted maxillary central incisors were divided into four groups of 13 each. Group 1: Sodium perborate + 3% Hydrogen peroxide, Group 2: Sodium perborate + 10% Carbamide peroxide, Group 3: 10% Carbamide peroxide +3% Hydrogen peroxide and Group 4: Control group (distilled water). Intra-coronal bleaching procedure was carried out in all the sample teeth and dentin microhardness was tested using Knoop indenter.

Result: One-way analysis of variance was carried out for testing significance of the differences in the mean values for multiple group comparisons. All results were considered statistically significant if $p < 0.05$. No significant difference in dentin microhardness was noticed in each group.

Conclusion: Hydrogen peroxide 3%, carbamide peroxide 10% and sodium perborate in the tested combinations are safe to use as intra-coronal bleaching agents in respect to their action on dentin microhardness.

Keywords: Carbamide peroxide; dentin microhardness; hydrogen peroxide; maxillary central incisors; sodium perborate.

INTRODUCTION

Discoloration in root canal treated teeth is one of the commonest problems and is of greater aesthetic concern. Among the bleaching methods intra-coronal bleaching is conservative simple and economical means to lighten root canal treated teeth.^{1,2}

Bleaching of endodontically treated teeth with walking bleach technique was described by Spasser

(1961)³ and modified by Nutting and Poe (1967).⁴ The most commonly used intra-coronal bleaching agents are hydrogen peroxide, sodium perborate, and carbamide peroxide.^{5,6} Studies on bleaching efficacy of different concentrations of carbamide peroxide and hydrogen peroxide as a bleaching agents have shown no statistically significant difference.^{3,6}

Although the intra-coronal bleaching process may provide satisfactory esthetic results, various studies

have pointed out that the agents applied in the pulp chamber may have some effects on microhardness^{7,8} biomechanical properties, ultrastructure and mineral content of dentin.^{9,10} Various studies have already been conducted for the evaluation of change in dentin microhardness with the use of intra-coronal bleaching agents.⁷⁻¹³ It has been shown in literature that the adverse effect of the bleaching agents on dentin microhardness is dose related.¹⁴

This study aims to evaluate the change in dentin micro-hardness using intra-coronal bleaching agents in combinations in lesser concentrations than in the previous studies.

MATERIALS AND METHOD

This comparative study was carried out in the Department of Conservative dentistry and Endodontics, Peoples Dental College and Hospital and Institute of Engineering, Mechanical laboratory, Pulchowk for the duration of one year.

Fifty-two extracted intact permanent maxillary central incisors were selected for the study. Teeth with caries, root canal treated and those with restorations were excluded from the study. Intact teeth extracted in periodontally compromised cases or for prosthetic purpose were included in the study. Convenient sampling was done for collecting and determining the sample size.

An approval letter of ethical clearance was obtained from Institutional review board, Institute of medicine, Maharajgunj before the research was performed.

All the selected teeth were thoroughly cleaned with an ultrasonic scaler and stored in distilled water until use. Teeth were decoronated two millimeters below cemento-enamel junction (CEJ) using diamond disc (Figure:1). The sectioning was done taking labial CEJ as a guide in each tooth. Samples

were divided into four groups consisting of 13 teeth each depending on the bleaching agents used.

Group 1: Samples to be bleached with Sodium perborate (A) and 3% Hydrogen peroxide(B)

Group 2: Samples to be bleached with Sodium perborate(A) and 10% Carbamide peroxide(B)

Group 3: Samples to be bleached with 10% Carbamide peroxide(A) and 3% Hydrogen peroxide(B)

Group 4: Control group in which distilled water was used

Endodontic access cavities were prepared using a round diamond bur, (Figure 2) and the pulp tissue from pulp chamber was removed with a spoon excavator. Apical orifice was sealed with 2 mm thick glass ionomer cement in each sample. (Figure 3).¹⁵

Preparation of 3% hydrogen peroxide: 100 ml of 6% hydrogen peroxide was taken on a flask and 100 ml of distilled water was added to the flask to make 200 ml of 3% hydrogen peroxide.

Preparation of bleaching agent: Bleaching agents in the respective groups were prepared in the ratio of 2:1 concentration (0.1g bleaching agent and 0.05ml vehicle).¹² The 2:1 ratio of bleaching agents was maintained between A and B bleaching agents respectively for each group. All the bleaching agents were measured in a weighing machine (Highland® portable precision balance) and using micropipette (Vertex) and mixed accordingly to obtain the required concentration Bleaching procedure: A fresh portion of bleaching agent prepared (respectively for each group) was placed in the pulp chamber of tooth sample in respective groups. Access cavity of tooth was sealed with Cavit due to its optimal sealing ability (figure 4).¹⁶ (Figure 4). After seven days Cavit was removed from each sample, the



Figure 1: Sectioning 2 mm below CEJ.



Figure 2: Access cavity preparation.



Figure 3: Sealing of apical orifice with GIC.

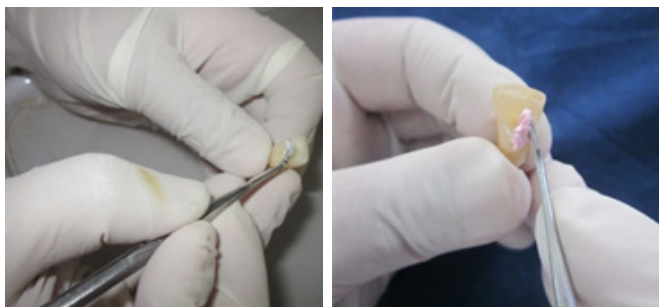


Figure 4: Bleaching agent placed followed by coronal sealing with Cavit.

bleaching agent was washed out with water and a fresh combination of respective bleaching agent was placed into the pulp chamber of each sample of each group. This procedure was repeated for three weeks. In control group, a cotton pellet soaked with distilled water was placed in the pulp chamber and replaced every week for three weeks. The sample teeth were wrapped in gauze, soaked with distilled water, and kept in an incubator (Veyance) at 37°C throughout the experiment to keep the samples hydrated as dentin hydration have been shown to affect dentin mechanical properties.¹⁷

After completion of bleaching procedure for three sessions each tooth was sectioned longitudinally (Figure 5) with the help of diamond disc and one half of each tooth was embedded in an acrylic mold (Figure 6). Uniform size and shape of acrylic mold were prepared using self-cure acrylic resin in a container. Samples were flattened and polished using 400,600 and 1200 grit SiC abrasive papers (Buehler).¹³

Detection of microhardness: A Knoop indenter was used, keeping the long axis of the diamond parallel to the dentin surface in a microhardness testing machine (Figure 7). For each specimen,



Figure 5: Longitudinal sectioning of tooth after 3 sessions of bleaching procedure.

indentation was made at dentin level using a load of 25 g for 15 seconds. Knoop microhardness (KHN) testing was done with a rhombic-based pyramidal diamond indenter that forms an elongated diamond shaped indent. The indenter was pressed into the sample by an accurately controlled test force (25 g) and maintained for a specific dwell time that is 15 seconds. After the dwell time was complete, the indenter was removed leaving an elongated diamond shaped indent in the sample. The size of the indent was determined optically by measuring the longest diagonal of the diamond shaped indent.

Dentin microhardness was calculated using the formula: $KHN = 14.22 P/l^2$, where P is the applied load (in kg) and l is the length (in mm) across the long axis of the remnant impression.¹⁸ For precision three different microhardness values of the same specimen were calculated.

Data were entered on SPSS version 20. Descriptive statistics were computed for all the variables. One-way analysis of variance (ANOVA) using F statistic of data were carried out for testing significance of the differences in the mean values for multiple group comparisons. All results were considered statistically significant if $p < 0.05$.



Figure 6: Preparation of sample for dentin microhardness testing



Figure 7: Indentation test in microhardness testing machine

RESULT

The calculated Knoop microhardness value of each sample in each group is shown in Table 1-4. For precision of the data, three values from each sample were calculated and mean was drawn. Mean value and standard deviation for each group is calculated and shown in Table 5. The maximum Knoop

microhardness value was observed for group II (69.25 ± 3.45) followed by group I (68.51 ± 1.51) and then by group III (68.22 ± 1.66). The microhardness value observed in all three groups are comparable with that of the control group IV (70.5 ± 3.60). Table 6 shows one-way ANOVA showing p value of 0.332, that is not significant.

Table 1: Microhardness values for group I samples.

Serial No.	Knoop Microhardness number (KHN)			Mean value
1.	66.5	68.2	67.8	67.50
2.	68.5	69	69.1	68.87
3.	69.5	68	68.5	68.67
4.	68	67	67.5	67.50
5.	70	69.5	69.7	69.73
6.	71	70.2	70	70.40
7.	68.8	70	69.2	69.33
8.	65	65.7	66	65.56
9.	68.1	68.5	69	68.53
10.	66	66.8	67	66.60
11.	70	71.2	71.5	70.90
12.	67	67.8	68	67.60
13.	69	69.2	70	69.40

Table 2: Microhardness values for group II samples.

Serial No.	Knoop Microhardness number (KHN)			Mean value
1.	69	68.5	66.2	67.90
2.	78.2	76.2	80.1	78.17
3.	67	68.2	68.2	67.80
4.	65.3	64.3	66	65.20
5.	66	68.8	68.8	67.87
6.	70	71.2	72.2	71.13
7.	72	74	76	74.00
8.	68	67.1	66.4	67.17
9.	67.4	67.8	65.8	67.00
10.	69.2	70.2	67.4	68.93
11.	67.4	68.4	70.2	68.67
12.	72.4	69.2	66.8	69.47
13.	65.2	66.2	69.4	66.93

Table 3: Microhardness values for group III samples.

Serial No.	Knoop Microhardness number (KHN)			Mean value
1.	70	69.2	69	69.40
2.	67	67.8	67	67.27
3.	67	66	65.8	66.27
4.	71.2	71.5	69	70.57
5.	69.7	70	69.5	69.73
6.	71	69.2	70.2	70.13
7.	69	69.1	68.5	68.87
8.	66.5	67.6	68	67.37
9.	68	66	67.5	67.17
10.	70	69.7	69.5	69.73
11.	67	66	66	66.33
12.	69	68.5	68.1	68.53
13.	66	65.6	64.8	65.47

Table 4: Microhardness values for group IV samples.

Serial No.	Knoop Microhardness number (KHN)			Mean value
1.	70	68.5	67.2	68.57
2.	78.2	77.2	82.3	79.23
3.	68.2	69.2	68.2	68.53
4.	65.3	66.3	66.4	66.00
5.	68	68.8	69.2	68.67
6.	70	72.2	74.2	72.13
7.	72	76	78	75.33
8.	69	68.2	67.4	68.20
9.	67.4	67.8	66.2	67.13
10.	65.2	68.2	72.2	68.53
11.	70.6	68.2	72.4	70.40
12.	70.2	69.2	68.4	69.27
13.	68.4	70.2	67.4	68.67

Table 5: Mean Microhardness and Standard Deviation values for each group.

Groups	N	Mean KHN±S.D
Sodium Perborate + 3% H ₂ O ₂	13	68.51±1.516
Sodium Perborate + 10% Carbamide Peroxide	13	69.25±3.454
10% Carbamide Peroxide + 3% Hydrogen Peroxide	13	68.22±1.667
Control (distilled water)	13	70.05 ±3.608
Total	52	69.0064±2.753

Table 6: One-way ANOVA test.

Microhardness	Sum of squares	Df	Mean Square	F	Sig.
Between Groups	26.271	3	8.757	1.167	0.332
Within Groups	360.340	48	7.507		
Total	386.611	51			

DISCUSSION

In this study, dentin microhardness was investigated after treatment with 10% carbamide peroxide, 3% hydrogen peroxide and sodium perborate in three different combinations. The microhardness value observed in all three groups are found comparable with that of the control group.

The mean dentin microhardness after bleaching with sodium perborate +3% hydrogen peroxide (group I) and the control group in present study was 68.51 and 70.05 KHN respectively. Contrary to the result observed in this study in group I, previous study by Oliveira in 2007¹² showed decreased micro-hardness value of dentin after bleaching with sodium perborate +30% hydrogen peroxide compared to the control group. Srivastav et al (2011)¹³ observed significant difference in microhardness value between control group and the group bleached with sodium perborate + 30% hydrogen peroxide. In vitro study by Pecora et al (1994)¹⁴ also observed slight decrease in dentin microhardness value after bleaching with 3% hydrogen peroxide + sodium perborate; from 46.9 to 44.4, from 46.5 to 45.6 KHN similar to the present study result.

The mean dentin microhardness value after bleaching with sodium perborate +10% carbamide peroxide (group II) and the control group in present study was 69.25 and 70.05 KHN, respectively. In vitro study by Oliveira (2007)¹² obtained the microhardness value for the group treated with 35% carbamide peroxide to be significantly different from the control group. In vitro study by Chng et al (2004)⁸ also showed significant decrease in microhardness value using 35% carbamide peroxide.

Consistent with the results of two tested groups the bleaching effect on dentin microhardness of group III bleaching agents (10% carbamide peroxide and 3% hydrogen peroxide) was not found significantly different from the control group.

Studies have indicated that hydrogen peroxide can cause dissolution of inorganic material, a reduction in the calcium-phosphorus ratio, and a reduction in the organic components of dentin by protein oxidation.^{19,20} Al-Salehi et al (2007)²¹ found that ion release from both enamel and dentine increased

with increasing hydrogen peroxide concentration in his study in bovine teeth. Rotstein et al 1992¹⁵ in his in vitro study concluded that 30% hydrogen peroxide treatment may cause alteration in the chemical structure of the dentin and cementum making them more susceptible to degradation. The pH of the bleaching agents, the buffering capacity of dentin, and the increase in diameter and density of the dentinal tubules as the pulp is approached are factors that can influence dentin microhardness.²¹ It is said that hydrogen peroxide affects not only the inorganic components of the dental hard tissues through acidic demineralization but also attacks the organic substance of the dentin. This effect of hydrogen peroxide on the organic substance might be due to collagen denaturation as proposed by Lado et al (1963).²²

Pecora et al (1994)¹⁵ tested different bleaching agents in combination for their effect on dentin microhardness. The bleaching agents tested in this study decreased human dentin microhardness. Endoperox (crystallized 35% hydrogen peroxide), Proxigel (10% carbamide peroxide) and 30% hydrogen peroxide greatly reduced dentin microhardness in comparison to the other groups. He has explained pH may be the cause of difference in the action of dentin microhardness by different combinations of bleaching agent. Chng et al (2004)⁸ in his study about role of bleaching agents on dentin microhardness has mentioned the unclear role of the pH of the bleaching agents. In the study it has been mentioned that other factors such as oxidation of the organic phase of dentine may be a responsible for the reduction in dentine microhardness.

Lewinstein et al (1994)⁷ has suggested that the use of high concentrations of hydrogen peroxide for bleaching purposes should be limited. Thus, in this in vitro study minimal concentration (3%) of hydrogen peroxide has been taken to evaluate the change in dentin microhardness after bleaching procedure.

Thus, comparing this study results with previous studies it can be said that there is significant decrease in dentin microhardness with increased concentrations of bleaching agents.

As the result has shown (Table 6) no any statistically significant difference in Knoop microhardness value for the tested three groups of bleaching

agents compared to the control group, the tested bleaching agent concentrations can be considered safe as intra-coronal bleaching agents. However, the complete clinical relevance of this study done in in vitro setup is difficult to ascertain. Further studies with a different study design are needed to find more evidence to accept or reject the null hypothesis.

ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to Mr. Hari Darlami, deputy head of department mechanical section, Institute of engineering, Pulchowk, for generously permitting me to use the microhardness testing machine in their laboratory for this study. I owe sincere thanks to Dr. Dipesh Tamrakar for helping me with the statistical analysis of this study. I am very much

grateful to my colleagues and all the assisting staffs in my department for their help during my research.

CONCLUSION

The dentin microhardness for the tested three groups (Sodium perborate and 3% Hydrogen peroxide, Sodium perborate and 10% Carbamide peroxide, 10% Carbamide peroxide and 3% Hydrogen peroxide) are not statistically significant to the Control group (Distilled water) and between the tested groups. Thus, it can be concluded that 3% Hydrogen peroxide, 10% Carbamide peroxide and Sodium perborate in the tested combinations are safe to use as intra-coronal bleaching agents in respect to their action on dentin microhardness.

CONFLICT OF INTEREST

No conflict of interest exists.

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