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Evaluation of Bleaching with Carbamide Peroxide on Microhardness, Mineral Content, and Color Change of White Spot Lesions

Abdolrahim Davari[1] ,Alireza Daneshkazemi[1] ،Farnaz Farahat[2] ،Elham Motallebi[3*] ،Sepideh Abbasi[4]

1. Department of Operative and aesthetic dentistry, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran/ Member of Social Determinant of Oral Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Operative and aesthetic dentistry, School of Dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Operative and aesthetic dentistry, School of Dentistry, Kashan University of Medical Sciences, Kashan, Iran
4. Department of operative and aesthetic dentistry, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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***Correspondence:**

Elham Motallebi,

Department of Operative and aesthetic dentistry, School of Dentistry, Kashan University of Medical Sciences, Kashan, Iran

elhammotallebi70@stu.ssu.ac.ir

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Abstract

***Background and Objective*:** Tooth bleaching changes the microhardness and mineral content and color of the tooth. The present study aimed to evaluate the effect of carbamide peroxide on microhardness, mineral content and color change in white spot lesions.

Material and Methods: Thirty-two samples were selected without caries, cracks and stains, and immersed in 0.5% chloramine-T for one week. Then the tooth crowns were separated. The teeth were artificially decayed by pH cycling. For bleaching on the enamel, a coating of 10% carbamide peroxide gel with a thickness of 1 mm was used for 8 hours. Samples were stored in artificial saliva for 16 hours. Bleaching process lasted for 14 days. Microhardness, color changes, calcium and phosphorus levels were measured before and after bleaching. Paired t-test and one-sample test were used to analyze the data.

Results: The microhardness test results were 338.02± 90.15 and 320.94±87.41 before and after bleaching, respectively. Microhardness of the samples significantly decreased after bleaching compared to before bleaching ($P<0.001$). Calcium and phosphorus content in samples after bleaching was not significantly different from before bleaching ($P>0.05$). The mean color change coefficient (ΔE) after bleaching was 6.82±3.96. ΔE in the studied samples was significantly higher than the standard ($\Delta E = 3.3$).

Conclusion: Bleaching with 10% carbamide peroxide significantly reduced microhardness. There was no change in the mineral content of the enamel and color change was proper. It can be concluded from this study that bleaching with carbamide peroxide can be successful.

Keywords: Tooth Bleaching [MeSH], Carbamide Peroxide [MeSH], Hardness Tests [MeSH]

Highlights

There is an association between the white lesions on changes in the mineral content of teeth and consequently, changes in tooth hardness, also Tooth bleaching changes the microhardness and mineral content and color of the tooth. Therefore in this study, evaluate the effect of bleaching with 10% carbamide peroxide on the color, microhardness, and mineral content of white spot lesions.

Introduction

Dental caries is one of the most common oral diseases worldwide, for which a multifactorial etiology has been suggested (1). Enamel decay occurs initially in the form of demineralization, decalcification, or mineral loss (2). Eventually, remineralization increases cavitation and porosity within the tooth structure, resulting in the appearance of a white lesion at this stage, called white spot lesions (WSLs) (3, 4).

When white spot lesions are exposed to substances in the oral environment, there is increased remineralization and mineral apposition on the surface layer. Minerals create a barrier, preventing the penetration of ionic materials into the white spot area, which causes optical changes in this area (4, 5). WSLs have a 40% prevalence in males and 30% in females (6). Color changes in demineralized enamel (in white spots) are similar to those after bleaching. These color changes are due to a decrease in the incidence of yellow and an increase in the incidence of white color (6- 8). The best approach for WSLs is a treatment that restores the physical (color) appearance and strengthens the weak structure of these lesions. Bleaching is likely to eliminate the white color resulting from WSLs. However, hydrogen peroxide bleaching reduces minerals in the teeth (calcium and phosphorus), especially white tooth lesions, which is a concern for dentists (9-11).

Other treatments have also been suggested for WSLs, each with advantages and disadvantages (1), including fluoride therapy, which is one of the most common and the oldest choices. However, this approach is not ideal, and the color changes do not completely disappear (12).

Despite great advances in tooth bleaching in recent years, there are still ambiguities about tooth bleaching that remain unanswered (1, 12). Besides, manufacturers of dental materials offer various new bleaching products due to the high benefits resulting from increased demand, which has particularly confused dentists in choosing the most appropriate ones (1, 12).

Many studies have been carried out on the effectiveness of bleaching agents based on carbamide peroxide and hydrogen peroxide. One of the shortcomings of previous studies might be evaluating the severity of color changes with the Vita color guide (13, 14). However, the standard color guide is not an accurate and reliable tool for evaluations (14).

Due to the high prevalence of dental caries and the effects of the associated white lesions on changes in the mineral content of teeth and, consequently, changes in tooth hardness and increased demand for removing these lesions, this study aimed to investigate the effect of bleaching with 10% carbamide peroxide on the color, microhardness, and mineral content of WSLs.

Materials and Methods

This study was performed using the lab-trial method with a parallel design and a combination of before & after. The samples in the present analytical study consisted of 16 human maxillary premolars extracted for orthodontic purposes without caries, cracks, and developmental defects were selected and evaluated under a stereomicroscope at ×10 magnification for the absence of white spots. Teeth with white spots, cracks or fractures, or any other lesions were excluded. After removing redundant tissues the teeth were immersed in 0.5% chloramine-T solution for one week for disinfection and stored at room temperature (15).Then the tooth roots were cut at the CEJ with a 0.2-mm diamond blade of a mechatronic (MPS203) cutting machine (Iran) after cleaning the teeth.

The middle third of the crown on the buccal and lingual aspects of the teeth was cut to a width of 2 mm, and the remaining occlusal and gingival thirds were discarded. Thus from each tooth, two enamel slabs measuring 6×4 mm from the buccal and lingual aspects of the crown middle third (Totally 32 sample) were prepared. The samples were stored in 37°C distilled water in a standard incubator for one week until all the samples were prepared. The slabs were then mounted in immediate acrylic resin molds with the enamel surface (6×4 mm) exposed.

- **Artificial White Spot**

To form artificial carries three pH cycles were used, which lasted for 12 days. Each 32 sample was immersed in 2.5 mL of demineralizing solution (1.5 mM CaCl2, 0.9 mM KH2PO4, 50 mM acetate buffer, pH=4.8,) for 72 hours, and then the samples were stored in 2.5 mL of remineralizing solution (1.5 mM CaCl2, 0.9 mM KH2PO4, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES], pH=7.0) for 24 hours at 4°C. This cycle was repeated three times (16).After to form artificial white spot, the samples were carefully polished with 240, 400, 600 grit silicon carbide papers. The samples were divided into two equal groups of sixteen before and after bleaching, then first stage of the test was performed to measure micro hardness, mineral content (calcium and phosphorus) and color on the sixteen of the samples before bleaching procedure.

- **Bleaching**

In the second stage, sixteen other samples were bleached, a layer of 10% carbamide peroxide gel (Opalescence PF 10%, Ultradent Company) with a thickness of 1 mm was applied, which remained on the enamel surface for 8 hours. Then the samples were rinsed with distilled water to remove the carbamide peroxide gel completely. The samples were then stored in an artificial saliva buffer made in the Faculty of Biochemistry with the formula of NaCl, CaCl2, KH2PO4 for 16 hours. This bleaching process lasted for 14 days.The pH of the bleaching gel used in this study was measured as 6.8. (16).

Then the samples of after bleaching procedure was performed to measure micro hardness, mineral content (calcium and phosphorus) and color.

- **Vickers Hardness Test**

This test uses a 136º diamond pyramid (Microhardness tester FM-700) to apply force to the cut tooth surface.

- **Color Change Detection Test**

Color change index was evaluated using an Easy Shade (VITA) tool. The color coordinates in this method were calculated with CIE*l*a*b before and after the bleaching process; then the values before and after bleaching were compared, and the color difference was reported as ΔE (16, 17).

$$\Delta E^* = [(\Delta l^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

- **Examination Of Mineral Content Using Electron Microscopy**

To investigate the mineralization, before applying the bleaching agent, the chemical composition and the percentage of elements in the dentin were measured in bulk at the surface of each sample by EDS-SEM (X-Pro, Switzerland).

- **Operating Conditions Of The Electron Microscope For Elemental Analysis**

Accelerator voltage = 15 kv, current = 10 nA, and time = 30-45 seconds with an operating distance of 10 mm. The electrons emitting from the electron gun

SPSS 23 was used for data analysis. Kolmogorov-Smirnov test was used to determine the normality of the data. If the data on microhardness and phosphorus and calcium content were distributed normally, paired t-test test was used, and if the data were not distributed normally, Wilcoxon non-parametric test was used. If color changes were distributed normally, one-sample t-test was used to compare the two groups; otherwise, Runs test was used to compare the groups.

- **Ethical Considerations**

The study protocol was approved by the Ethics Committee in Biomedical Research, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, under the code IR.SSU.REC.1398.111.

Result

The microhardness test results before and after bleaching were 338.02±15.90 and 320.94±41.87, respectively ($P<0.001$).

The calcium measurement test result values before and after bleaching were 66.2±10.62 and 65.95±2.14, respectively ($P=0.113$) (Figure 1).

Besides, the phosphorus measurement test result values for before and after bleaching were 35.3±14.35 and 34.03±1.75, respectively ($P=0.09$) (Figure 2).

The mean color change coefficient (ΔE) in the present study was 6.82±3.93. One-sample t-test was used to compare this rate with the standard score of color change (Figure 3).

As shown in Table 1, ΔE in the samples in the present study is significantly higher than the standard score.

The amount of ΔE in seven samples (21.9%) was lower than the standard color change score, and 25 samples (78.1%) exhibited higher scores than the standard score.

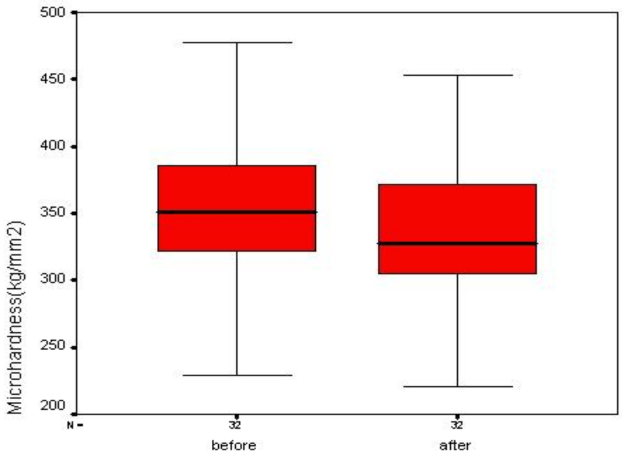

Figure 1. Comparison of samples’ microhardness before and after bleaching.

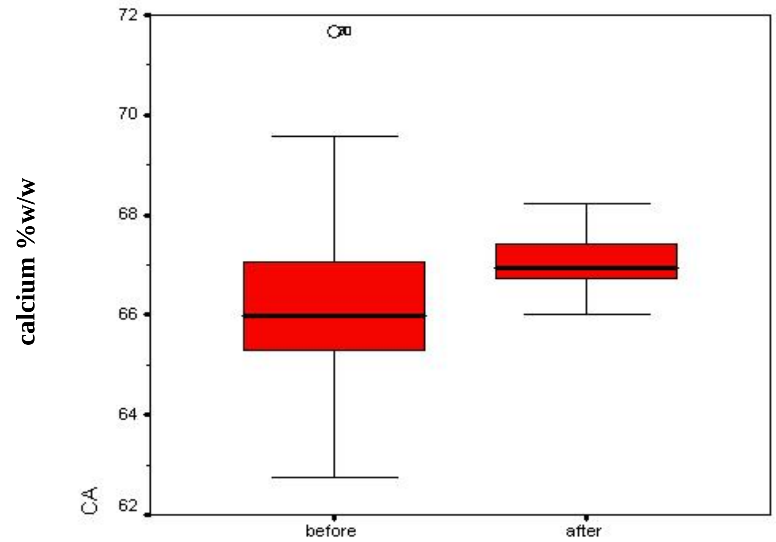

Figure 2. Comparison of samples' calcium levels (%w/w) before and after bleaching

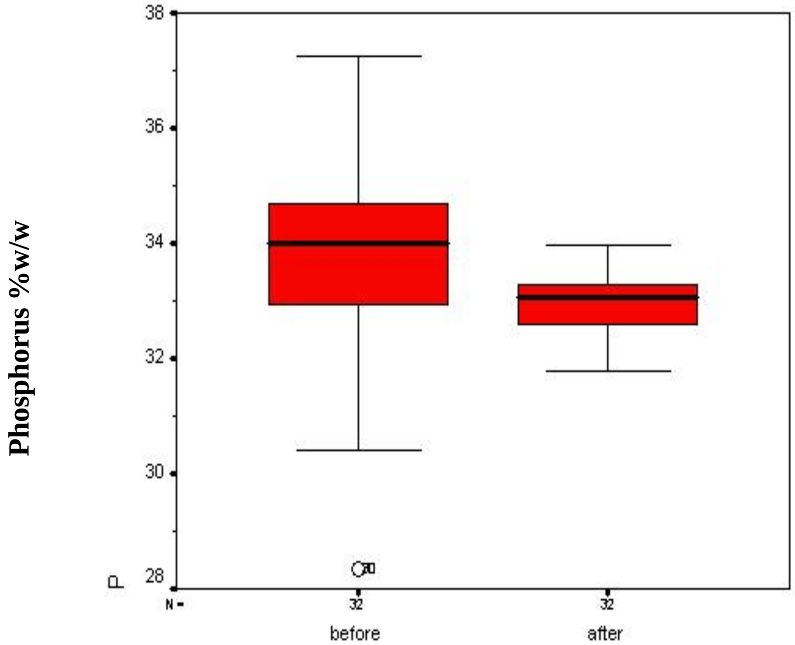

Figure 3. Comparison of samples' phosphorus levels (%w/w) before and after bleaching

Table 1. One-sample t-test was used to compare the color change score in the sample group with the standard score

Variable	Mean difference	SD	Standard score	T	P
Δ E	3.51	1.79	3.3	5.2	P<0.001

Discussion

As mentioned previously, there is significant disagreement about the effect of bleaching on tooth microhardness, which might be due to several factors, including different substrates (bovine or human teeth or even composite materials), how to determine microhardness, different bleaching times and storage, different caries induction processes, study type (in vitro or in vivo), different types and percentages and combination with other bleaching methods (18). In addition the pH of the bleaching agent is also factor that can directly influence the whitening process. At the present, manufacturers have shown concern about the acidity of bleaching gels. Because a pH below the critical point (pH 5.5 to 6.5) may result in the dissolution of enamel and micro hardness alterations (19). Thus the pH of the bleaching gel used in this experiment was measured as 6.8.In the present study, no significant differences were observed between the groups. The Vickers hardness test was employed in the current study. The diamond indenter tip of the Vickers machine penetrates deeper layers of enamel, which might not have been affected by tooth bleaching, and the hardness of the area might be the same as unbleached enamel (20). The remineralization and demineralization phenomena increase and decrease the enamel microhardness, respectively (18).

Since in the present study, a change was observed in the studied groups' microhardness, it seems that the enamel demineralization process was not compensated by the effective factors in remineralization in artificial saliva. Therefore, it seems that combining the bleaching process with substances that increase remineralization is a favorable method to increase microhardness. The demineralization process begins with the loss of mineral content of teeth, especially calcium and phosphate from hydroxyapatite crystals in hard dental tissue; in a healthy individual, this process can be compensated by remineralization (19). Laboratory studies have also shown that the bleaching process with carbamide peroxide changes the mineral content of teeth (20-22). These changes are likely to decrease calcium, phosphorus, and fluoride contents (23-25). However, in another study, it was suggested that bleaching with 10% carbamide peroxide causes a slight and insignificant reduction in the mineral content of the tooth (26).

To reduce the loss of dental mineral content during the bleaching process, the manufacturers of bleaching agents add various compounds, including fluoride ions and amorphous calcium phosphate, to their products (27-29). It has also been reported that incorporating fluoride ions and other minerals in the oral environment increases remineralization (28, 30). Calcium ion also makes the tooth resistant to an acidic environment and prevents the adverse effects of the bleaching process (31, 32). However, studies have shown that the bleaching process does not affect the process of demineralization in bovine teeth (33).

The present results showed that the amount of calcium and phosphorus in the teeth did not change significantly after the bleaching process; however, these values decreased slightly [Ca (P=0.09) and P (P=0.113)].

Consistent with the present study, Tezel et al (2013) reported that the bleaching with 10% carbamide peroxide did not change the calcium content of bleached teeth. However, some demineralization was observed in bleaching with 35% and 38% hydrogen peroxide. One of the notable results of this study was a greater increase in calcium loss during long bleaching procedures (21). The similarity of the study with ours was in the use of carbamide peroxide in the bleaching process. However, the method used to measure minerals in the present study was different, making our results more valid.

Amaral et al (34) examined the amount of calcium and phosphorus in enamel after different bleaching processes carried out using 35% and 38% hydrogen peroxide and 10% and 20% carbamide peroxide both at in-home and in-office bleaching. Phosphorus was measured before, after, and during the bleaching period. Their results showed that the amount of phosphorus before and after bleaching was not different, and also, this amount was not related to the length of the bleaching period. It was also shown that the highest amount of phosphorus was observed with bleaching with carbamide peroxide, and the lowest was observed with 38% hydrogen peroxide. Their results showed that the amount of calcium after bleaching was not significantly different from the amount before treatment. The lowest amount of calcium in enamel was related to 38% hydrogen peroxide, which was significantly different from this amount using 10% carbamide peroxide. However, there was no significant difference from 20% carbamide peroxide (32). According to the present study and Amaral study results, it can be claimed that different bleaching processes have different effects on the amount of phosphorus and calcium, and it seems that the best bleaching method for preserving enamel minerals is the use of 10% carbamide peroxide (34). However, more studies are necessary to substantiate this claim.

Santini et al (2008) showed that bleaching with carbamide peroxide reduced the amount of phosphorus in enamel at 7, 14, and 21 days after bleaching compared to that before bleaching (35). The difference between the results of the present study and the study above can be attributed to the number of samples and the method used to determine the amount of phosphorus. The study by Santini et al was performed on only six samples; however, the number of samples in the present study was more than five times higher. They also used Raman infrared spectrophotometry to determine the amount of phosphorus, which was different from the technique used in the present study.

Faraoni-Romano et al also concluded that the use of 10% carbamide peroxide for five weeks did not result in the surface morphology changes, etching, and decalcification of the enamel (36).

Saliva can play a remineralizing role and maintain the oral environment conditions after bleaching to improve the tooth structural properties (2, 9). Potential salivary remineralizing factors are calcium and phosphate ions (35).

The best treatment for these lesions is the treatment that restores the physical (colored) appearance and strengthens the weak structure of these lesions. The discoloration in demineralized enamel (in white lesions) is similar to the discoloration resulting from bleaching. These color changes are due to adecrease in the incidence of yellow color and an increase in the incidence of white color (7, 8). Bleaching is likely to eliminate the white color from white tooth lesions.

Carbamide peroxide is a powerful oxidizing agent with a high ability to penetrate tooth structure, especially enamel, causing the formation of free radicals (H2O-) and superoxide (O2-). Eventually, these highly reactive radicals attack the chain of macromolecules that cause discoloration and break them down into smaller pieces, removing discoloration (36). Physically, this process can be largely explained by the fact that free radicals, in addition to the decomposition of macromolecules in chain reactions, produce other organic radicals that attack other double and unsaturated bonds and change the electron distribution at the atomic level, causing a change in the molecular adsorption of these macromolecules. Therefore, it can be claimed that with these changes, less light is reflected from the tooth surface, making it brighter (37).

The present study results showed that tooth bleaching with 10% carbamide peroxide (Opalescence PF) resulted in more color changes than the standard criterion. It should also be noted that more than 78% of the samples had color changes above the standard level. It should be noted that the use of artificial saliva and the bleaching process similar to that in the clinic were among the advantages of the present study so that it significantly simulated the clinical condition.

Zekonis et al showed that individuals who used 10% carbamide peroxide to eliminate WSLs were approximately 5.5 times more satisfied than those who used 35% hydrogen peroxide (38). Also, the ΔE for carbamide peroxide in their study was 4.3. The difference between this value and tha t in our study (6, 24) could be due to the bleaching process; they performed the bleaching in the patient's oral environment, whereas our study was in vitro.

Consistent with the present study, Goo et al (39) in 2003 studied the effect of 10% carbamide peroxide on color changes of bleached teeth. They reported that carbamide peroxide dramatically changed the enamel color over time, whereas no change was observed in the other group. The mean ΔE in the bleached group after 14 days was 5.53, which was lower than that in the present study. Such difference might be attributed to storing the teeth in artificial saliva in the present study.

Kim et al (2016) evaluated the effect of bleaching on color changes and chemical and mechanical properties in white spot lesions. They reported that the best color changes were related to 10% carbamide peroxide (16).

According to the present study and the previous studies, it could be concluded that bleaching with carbamide peroxide, especially Opalescence PF, can be successful in the clinic.

Conclusion

The results showed that bleaching with 10% carbamide peroxide resulted in microhardness reduction, no changes in the enamel mineral content, and proper color changes. According to this study ‹whitening regimen for white spot lesions is a noninvasive esthetic treatment that was recommended.

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