Effects of Different Bleaching Methods on the Enamel Mineralization Level

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ABSTRACT
Objective: The aim of the present study is to evaluate the mineralization level of human enamel following bleaching treatment using a laser fluorescence device.
Methods: A total of 45 patients were divided into three groups according to the bleaching method used. In Group 1, chemical bleaching was conducted with 35% hydrogen peroxide (HP). In Group 2, we used 35% HP activated by a 980 nm diode laser. In Group 3, bleaching was conducted with ozone. Levels of mineralization were measured using a laser fluorescence device before bleaching, just after bleaching and also on the 2-weeks and 1-month following bleaching.
Results: Data were analyzed using the Kruskal–Wallis test, one-way analysis of variance, and Mann–Whitney U test. The values were significantly higher compared to initial values following bleaching and at the 2-weeks follow-up for Groups 1 and 2 (p<0.05). However, values were similar to the initial values at the 1-month recall (p>0.05). For Group 3, the values were significantly reduced following bleaching (p<0.05), while the values of each postbleaching were statistically similar (p>0.05).
Conclusion: Both chemical and laser-activated bleaching resulted in a significant reduction of the mineralization level, while bleaching with ozone did not. Bleaching with ozone seems to be safer in terms of demineralization compared to HP.
Keywords: Dental bleaching, DIAGNOdent, diode laser, ozone treatment

INTRODUCTION
Office bleaching methods have been widely used to whiten discolored vital teeth. Hydrogen peroxide (HP) at high concentrations is the most used agent for this purpose and can be arbitrarily activated by heat or light to obtain better results. This chemical leads to whitening by oxidizing organic and inorganic compounds. However, the exact mechanism of bleaching by HP is not well known. The degree of oxidation depends on environmental conditions including temperature, pH, and light. For this reason, different activation methods such as light and lasers have been used to heat HP and in this way accelerate the decomposition of oxygen to form more oxygen-free radicals (1). On the other hand, both chemical bleaching and laser-light-activated techniques have been probed due to their deleterious effects on the tooth structure (2). A previous study revealed that the structural alteration may occur in the enamel following bleaching, which is directly proportional to the concentration of HP and which result in a reduction of the enamel microhardness as a result of demineralization and loss of mineral content (3, 4).

The ozone application is a contemporary technique used for various dental treatments, including remineralization of dental hard tissues and management of dental hypersensitivity (5). This method has recently been used for bleaching to avoid undesired effects of HP (6). Bleaching with ozone acts by oxidizing. For this reason, bleaching with ozone became an alternative to bleaching with HP (7).

The DIAGNOdent device reflects the 655 nm monochromatic light to a biological substrate and recollects scattered light to detect any deviations on surfaces (8). It was first manufactured for the purpose of an early detection of the degree of demineralization (9).

It uses a scoring range from 0 to 99 as follows:
0–13: No cavities
14–20: Demineralization of enamel
21–29: Demineralization of superficial dentin
30–99: Demineralization of deep dentin (10)

The present study compared demineralization following different bleaching methods, including chemical bleaching with HP HP activated by laser, and bleaching with ozone. The level of demineralization was measured using a DIAGNOdent pen (Kavo, Biberach, Germany).
METHODS

The present study was approved by the ethical committee of Gaziantep University (2018/140).

Inclusion criteria were the following:

1. The presence of all maxillary and mandibular incisors, canines, and first premolars.
2. C1 or darker color shades of the relevant teeth.
3. Absence of any restoration that may interfere with the DIAGNOdent pen readings.
4. Participants with good oral hygiene were included.
5. Systemic health status of patients was also considered to be normal.

Patients with poor oral hygiene, pregnant or lactating women, and individuals who were smoking or chewing tobacco were excluded.

A total of 45 patients were divided into three groups according to the bleaching protocol (n=15).

Before the bleaching procedure, the prophylaxis paste was applied to relevant teeth. Initial measurement with the DIAGNOdent pen was performed (Figure 1). Gingival tissues were isolated using a light-polymerized resin dam (Top Dam; FGM Prod., Joinville, SC, Brazil).

The bleaching procedures were conducted as follows:

Group 1: Bleaching gel containing 35% HP (Whiteness HP BLUE CALCIUM- FGM, Joinville, SC, Brazil) was coated to dried facial enamel surfaces at a thickness of 1.5 mm. The gel was kept on the teeth surface for 15 minutes and removed with air/water spray. This was repeated three times.

Group 2: Bleaching gel containing 35% HP (Whiteness HP BLUE CALCIUM-FGM, Joinville, SC, Brazil) was coated to dried facial enamel surfaces at a thickness of 1.5 mm. The gel was kept on the teeth surface for 15 minutes. During this 15-minute period, laser activation of the bleaching gel with a diode laser (980 nm, 4 W) for 2 minutes (30 seconds for each quadrant) was further achieved. For this purpose, a special bleaching handpiece was...
attached to the laser device, and irradiation was applied 6 mm away from the relevant teeth (Figure 2). Then, remnants of the gel were removed with air/water spray. This was repeated two times.

Group 3: Cast models were constructed for each patient’s maxilla and mandibular and custom trays made from thermoplastic material (Easy-Vac Gasket, 3A MEDES, Gyeonggi-do, Korea) fitting both jaws of patients were prepared. The hose pipe of the

Figure 2. a, b. (a) Laser-activated bleaching; (b) Laser activation handpiece (Devices in Figures belong to Archive of Gaziantep University School Medicine - used with permission)

Figure 3. a, b. (a) Ozone generator device; (b) Intra-oral application of ozone for bleaching (Devices in Figures belong to Archive of Gaziantep University School Medicine - used with permission)
ozone-releasing machine (Ozonyton XP-OZ MIO international, Munich, Germany) was attached to the custom tray, and ozone was applied to the external surfaces of teeth by using a special setting for bleaching (600,000 ppm). Ozone was applied to each arch for 15 minutes twice (Figure 3).

The second, third, and fourth measurements were performed just after bleaching, at the 2-weeks recall and at 1-month recall, respectively, by using the DIAGNOdent pen. The differences of values at each measurement with the initial measurement were recorded.

**Statistical Analysis**

Data were shown as the median, interquartile range, or frequency and percentage. Prior to statistical analysis, the normality of data was analyzed with the Shaprio–Wilk test. Due to the non-normal distribution of the data, statistical analysis was performed using the Kruskal–Wallis one-way analysis of variance followed by the Mann–Whitney U test. The Kruskal–Wallis and Mann–Whitney U tests were used to compare the variables within the groups. (intergroup comparisons were not performed) A p-value <0.05 was considered statistically significant. The analysis was performed using the Statistical Package for the Social Sciences program version 19 (SPSS IBM Corp.; Armonk, NY, USA).

**RESULTS**

Mean values obtained for each group and time intervals are presented in Table 1 and graphically presented in Figure 4.

Both the chemical and laser-activated treatment (Groups 1 and 2) resulted in significant demineralization following bleaching and at the 2-weeks follow-up (p<0.05). However, values were similar to the initial values at the 1-month follow-up (p>0.05).

In the ozone group (Group 3), the samples showed significant remineralization following bleaching (p<0.05). Values at each follow-up visit were statistically similar (p>0.05). Ozone did not lead to any demineralization.

**DISCUSSION**

Bleaching is a cosmetic dental application that has gained popularity in recent years. Several materials and techniques have been used for this purpose. HP is one of most preferred bleaching agents. It owes its whitening effect to the oxidation reaction that destroys discoloring molecules (11).

The by-products generated following oxidation of HP can easily penetrate to enamel and dentin (12). However, the exact mechanism of bleaching with HP has not been fully understood yet because the question as to which inorganic or organic phases of the dentin and enamel are affected has not been clearly answered. On the other hand, this HP mechanism of action is also suspected to cause some damage to dental hard tissues. Demineralization is a process that involves the loss of calcium ions in the surface of calcified dental tissues (13). A previous study by Rotstein et al. (14) revealed that 10% carbamide peroxide and 30% HP resulted in a significant reduction in the calcium-to-phosphorous ratio of dentin and caused mineral loss following bleaching. However, another study found that HP does not affect the inorganic phase and that it only destructs the organic components (11). According to the results of our study, HP-utilizing bleaching protocols (Groups 1 and 2) represented a significant decrease in the degree of the mineralization of enamel (15). Considering the results of the previous studies, we assume that this is correlated with the change in the inorganic enamel content. However, the mineralization level returned to a value close to the initial 1 month later. This is probably related to the saliva remineralization effect (16). In other words, it seems that the demineralization caused by HP is reversible. Nevertheless, dietary habits and oral care should be attended by patients during this temporary phase because of the tendency of cavities development. Potočnik et al. (17) reported that bleaching agents may lead to microstructural damages on the enamel surface.

| Table 1. Distribution of mineralization values following different bleaching methods |
|---------------------|---------------------|
| **Groups** | **Measurement time** | **Mean±standard deviation** |
| | | |
| **Group 1** | Initial | 2.65±0.18<sup>a</sup> |
| | After bleaching | 5.48±0.85<sup>b</sup> |
| | After 2 weeks | 4.15±0.22<sup>b</sup> |
| | After a month | 2.91±0.18<sup>b</sup> |
| **Group 2** | Initial | 1.87±0.17<sup>a</sup> |
| | After bleaching | 3.75±0.35<sup>b</sup> |
| | After 2 weeks | 3.15±0.21<sup>b</sup> |
| | After a month | 2.25±0.19<sup>b</sup> |
| **Group 3** | Initial | 4.63±0.41<sup>a</sup> |
| | After bleaching | 2.56±0.33<sup>b</sup> |
| | After 2 weeks | 2.05±0.13<sup>b</sup> |
| | After a month | 1.96±0.1<sup>b</sup> |

Different superscript lowercase letters indicate statistical difference for intra-group comparison.
such as initial cavity lesions. Demineralization of the outer enamel means the reduction of enamel microhardness, which makes teeth less resistant to cavities (3).

Bistey et al. (4) reported that demineralization following teeth bleaching is directly proportional with the time of application and concentration of the agent. In the present study, HP was used at a concentration of 35%, which is commonly used for a bleaching treatment at a range between 35% and 40%. Duration of chemical bleaching for such agents is approximately 45 minutes according to the manufacturers’ recommendations. Furthermore, the activation of chemical agents with lasers is performed to lessen the duration of treatment (18). However, in the laser-activated group (Group 2) of the present study, although HP was applied for 15 minutes less than the chemical bleaching group (Group 1), the results of both groups were similar in terms of demineralization values. This may be related with the action of mechanism during the activation of bleaching materials with a laser, which accelerates the formation of free oxygen radicals. Although this lessens the duration of the bleaching treatment, the amount of free oxygen radicals is similar to chemical bleaching alone (19). This may explain why both groups revealed a similar degree of demineralization. However, both groups returned to similar values compared to the initial ones. Justino et al. (16) reported that a 2 weeks contact with saliva provides enough enamel remineralization following bleaching. In the present study, the time required for remineralization was 1-month. This difference may be related to agents which concentration and duration of application varied.

Various techniques including visual inspection, photographic examination, fluorescent dye uptake, ultraviolet light, and laser fluorescence were used to determine the degree of enamel demineralization (20). In recent years, DIAGNOdent have also been used to detect the mineralization status of dental hard tissues (21). The device gives measurements ranging between 0 and 99. Low values indicate high mineral content, which means that the tooth structure is healthy.

Ozone is another material used in many dental procedures. Particularly, it was employed for bleaching due to its gaseous (O2) structure (22). The oxidizing ability of ozone breaks down the chromogen molecules similar to HP and thus constitutes an alternative bleaching method (23). Requiring no light activation, lower cost and less irritation are the advantages of bleaching with ozone compared to HP (24). According to the present study results, another advantage of bleaching with ozone seems to be its remineralization effect contrary to HP. This may be related to findings by Valko et al. (25), who reported that different free radicals were produced with two different materials. Ozone produced oxygen radicals, while HP released HO2 free radicals that are stronger than oxygen radicals. In fact, previous studies found that ozone increases remineralization in accordance with our results (26). Furthermore, Baysan and Lynch reported that ozone removes the proteins from the demineralized regions of the enamel and thus accelerate the penetration of calcium and phosphate ions (27). Furthermore, another study found that ozone provides an alkaline environment and induces remineralization using minerals from saliva (28). These statements may further explain why the ozone group of the present study did not show demineralization, but on contrary, it showed an increased degree of remineralization.

CONCLUSION

Within the limitations of this study, it was concluded that bleaching with ozone seems to be a safe bleaching method due to its remineralization effect and cavities prevention.

Ethics Committee Approval: The present study was approved by the Ethical Committee of Gaziantep University (2018/140).

Informed Consent: Written informed consents were taken from the patients.

Peer-review: Externally peer-reviewed.

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