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Evaluation of teeth whitening with application of novel toothpaste containing ozone

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Original Article

Abstract

BACKGROUND AND AIM: The aim of this study was to evaluate the effectiveness of different whitening toothpastes, focusing on the experimental ozonated toothpaste.

METHODS: This laboratory study included a sample of 48 sound human molar teeth. Teeth were randomly assigned into four groups according to toothpaste treatment to be evaluated objectively (colorimetric method) and subjectively (visual assessment) (n = 12). Group I: Ozonated toothpaste (experimental); Group II: Non-ozonated toothpaste (experimental); Group III: Aqua fresh whitening triple protection; and Group IV: Nasim (toothpaste without a chemical whitening agent). After tea staining and color assessment, the teeth were subjected to a tooth brushing regime as for 6 weeks, done twice a day, 2 min each time (total: 168 min). Next, color changes were determined after brushing by instrumental and visual methods.

RESULTS: Analysis of variance and the Tukey tests were used for evaluating statistical data ($\alpha = 0.05$). Color change by instrumental index showed that ozonated and aquafresh toothpastes increased teeth whitening; however, the amount color change was not significant ($P > 0.050$). With visual assessment there was a significant difference between mean color change among the four groups ($P = 0.008$).

CONCLUSION: Ozonated toothpaste caused significant whitening changes in discolored teeth from a clinical point of view by visual assessment.

KEYWORDS: Ozone, Toothpaste, Whitening

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People have a strong desire to have white teeth and many patients are dissatisfied with their current tooth color as indicated in a number of recent studies.¹ As a result, nowadays, clinical professional treatments are available to patients in conjunction with daily oral hygiene tools such as various toothpastes meant to remove certain types of dental discoloration. These toothpastes contain chemical or abrasive whitening compounds. There are many methods to improve tooth color, namely tooth bleaching or the removal

and control of extrinsic stain and placement of esthetic restorations.²

The introduction of different formulations of peroxide and their product variety, such as gels, rinses, gums, mouthwash, strips, and coloring agents, are available over the counter in pharmacies, as well as being conveniently available from the internet. They are alternative solutions for at-home dental whitening.³ Tooth color is dependent on the color of dentin and on internal and external absorbed stains.^{4,5} Any change in the structure of enamel, dentin and pulp of a

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tooth crown can cause changes in the way light passes through the tooth and hence, result in tooth color changes.⁶

It is very important to determine the essential etiology of tooth discoloration for conducting a successful treatment. Changes in tooth color can be divided into two main groups of internal and external or a combination of both.⁴ The most common reason of external color changes are dark foods, usage of some medications, tobacco use, certain types of bacteria in oral flora and the presence of pits, fissures, cracks or defects in the enamel.^{7,8} Some extrinsic stains that remain on the tooth for a long time become intrinsic. The removal and control of extrinsic stain is possible with toothpaste and in particular, tooth whitening formulations, which typically contain optimized chemical and abrasive ingredients to maximize cleaning.⁹ The whitening effects of chemical ingredients can be observed quickly after 4-7 days, whereas abrasive agents show their influence over a longer period (2-4 weeks).¹⁰

Scientific support, as suggested by demonstrated studies, for ozone therapy presents a potential for an a traumatic, biologically-based treatment for conditions encountered in dental practice and that ozone can be successfully used for lightening the yellowish tinge of tetracycline-stained rat incisors.^{11,12} Hence in this study, special ozonated toothpaste was used in order to whitening teeth. The evaluation of whitening toothpaste on tooth color changes can be measured with a colorimeter, spectrophotometer or by comparison with a vita shade guide under controlled lighting conditions.⁹ According to the best of the authors' knowledge, no one employed in trial an ozonated toothpaste as a teeth whitening agent. The aim of this study was to evaluate the effectiveness of different types of toothpaste products in teeth whitening, with a focus on the experimental ozonated toothpaste by the colorimetric and visual methods.

Methods

This laboratory study was conducted on 48 recently extracted sound human molar teeth under a protocol approved by the Ethics Committee of the Mashhad School of Dentistry, Mashhad University of Medical Sciences, Iran (87892/2009). Formalin solution 10% was used to disinfect the teeth. First, an ultrasonic cleaner and then rubber cup with the prophylaxis paste by low speed handpiece were used for 2 min to remove debris and stains on teeth crown surfaces. Next, all teeth were examined for any cracks and decay due to restorations, crown color change and other possible defects. Then, the roots were cut by a disc from the cemento-enamel junction and good quality clear nail polish was used to seal the crown bottoms and then were mounted in immediate self-cured acrylic (Akro Pars, Iran).

Each sample was mounted in the center of a cylinder and labial surfaces of all teeth were placed toward the outside with about 1 mm of the labial surface remaining out of the acrylic. These cylinders were selected based on the holes' diameter of the artificial tooth brush device so as to be able to place the mounted samples in the holes. Each tooth was immersed separately in cans containing standardized black tea bag (Golestan, Iran Co) solution for 2 weeks using the method described by Sulieman et al.¹³

The tea solution was prepared by boiling 2 g of black tea with 100 ml of distilled water for 5 min and then filtered to remove the tea leaves from the infusion. In order to simulate the oral environment, the samples were kept inside an incubator at 37 °C while being immersed in tea solution. During this time, the tea solution was changed daily. After tea staining, since the purposed was to evaluate tooth whitening in where the toothpaste has been designed to have an effect on the average intrinsic tooth paste color, the teeth were thoroughly polished to remove any traces of surface extrinsic stain. This was then followed by a brushing protocol with the

tested toothpastes.

Teeth were randomly divided into four groups of 12 teeth each as follows: Group I: Ozonated toothpaste (experimental; the peroxide concentration was equivalent to 25-50 mmol/g of H₂O₂), Group II: Non-ozonated toothpaste (control), Group III: Aqua fresh whitening triple protection toothpaste, Group IV: Nasim (toothpaste without any chemical whitening agent). Tooth brushing was done by an electric device (Nemo, Mashhad, Iran). Teeth color measurements were conducted in two stages; first, after tooth staining (Stage T1), and immediately after tooth brushing (Stage T2) with different types of toothpastes. The shade of each tooth was measured by the colorimetric method and visual assessment.

Objective color assessment of samples was done by the colorimeter color eye (XTH, X-rite, Grand Rapids, MI, USA). The surface of each tooth was covered with an acid-resistant nail varnish, leaving a window of approximately 4 mm × 4 mm at the center of the buccal surface exposed. This device was used under the same light conditions and the evaluator held the same position during the two stages for all of the teeth. The samples were completely dried by cotton before colorimetric assessment.

In this study, the standard light source was D65 and the whiteness index was E313. The device was calibrated by the device's enclosed white pill before conducting the color assessment and then the desired sample was placed under the diaphragm at the light source. All the color changes between the two-stages of color assessment were recorded as L* (lightness), a* (redness), and b* (yellowness) axes based on the CIE Lab (Commission International de l'Éclairage) system. An L* value indicates the lightness of tooth samples and the range is from 0 = black to 100 = white, whereas a*, and b* values indicate positions on red/green (+a = red, -a = green) and yellow/blue (+b = yellow, -b = blue) axes, respectively. Total color

difference (ΔE) was calculated by the following formula:

$$\Delta E^2 = \Delta a^2 + \Delta b^2 + \Delta L^2$$

Standard vita shade guide (Vita, Zahnfabrik, Germany) tabs that were arranged from B1 to C4, corresponding to a grade of whitening from 1 to 16 was used. Although this scale is not linear in the truest sense, the changes were treated as though they represented a continuous and approximately linear ranking for the purpose of analysis. A trained evaluator conducted the visual evaluation and repeated measurements consequently 2 times for each tooth in middle of sunny days (10 am). Initially, the teeth were placed on a dark background to simulate the dark oral environment. Next, color assessment was performed under a uniform and constant light environment in the laboratory for all samples that were done in two-stages. Vita color differences were calculated by the following formula:

$$\Delta \text{ Vita shade change} = \text{Vita score (treatment)} - \text{Vita score (baseline)}$$

The ozone used in this study was produced in the pharmacology research laboratory by an American made ozone-generating machine with the ability to produce 13 g of ozone per hour. Due to the unstable properties of ozone, ozone gas was blown into an olive oil tank by a pipe at a speed of 13 g/h to make ozonated olive oil. Then, the olive oil was converted into a gel with full oxidization properties after 48 h periods to be used as toothpaste in group I of this study.

The pH 8.5 of ozonated olive oil was obtained by adding 1 m sodium hydroxide solution and then sodium lauryl sulfate was added to reach a 0.1% concentration. The tooth brushing regimens were performed equally for 6 weeks, twice a day and each time for 2 min. For the artificial brush system, the total period of brushing was equivalent to 168 min. Equal amounts of toothpaste were used for all samples after

every 2 min, fresh toothpaste was placed on the surface of the teeth and the surfaces were kept moist during brushing. When the samples were not in use, they were stored in water and away from light. Immediately after brushing with the four toothpastes, the sample colors were evaluated by colorimetric and visual assessments and data was recorded. One-way analysis of variance (ANOVA) and Tukey tests were used to analysis the color data that were obtained by colorimeter and visual assessments. Significant levels were considered to be $\alpha = 0.05$.

Results

In colorimetric assessment, from one stage to the next, the mean L^* value showed a significant increase (Figure 1).

From Stage I to II the mean a^* value decreased in all groups, except for the non-ozonated toothpaste group; however, this reduction was significant only in the aqua fresh group (Figure 2).

In all groups, except for the non-ozonated toothpaste group, from Stage I to II the mean b^* value decreased and this value was significant in the ozonated and aquafresh dentifrice groups (Figure 3).

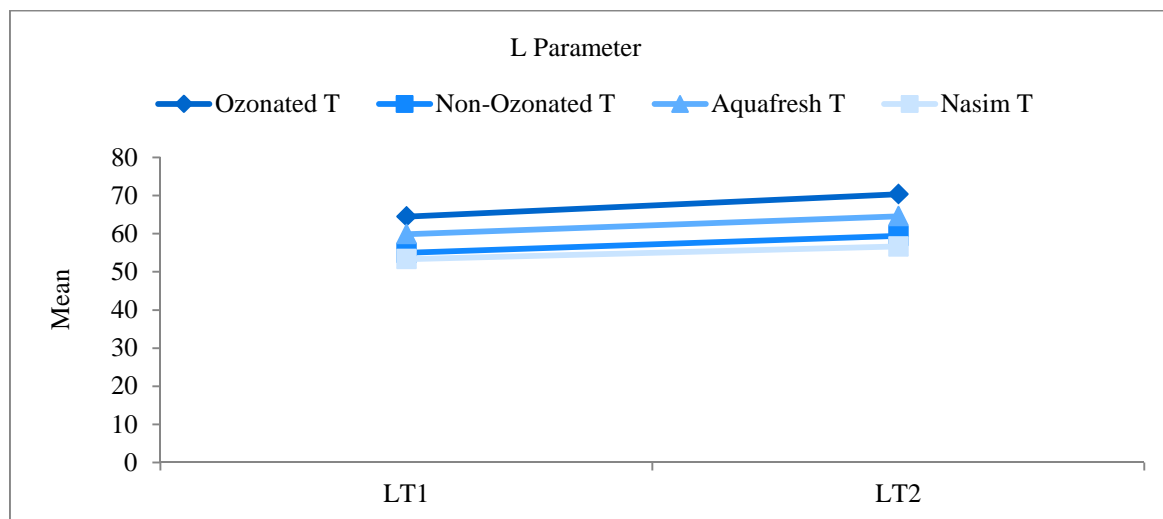


Figure 1. Mean of L parameter changes before and after brushing with different toothpastes

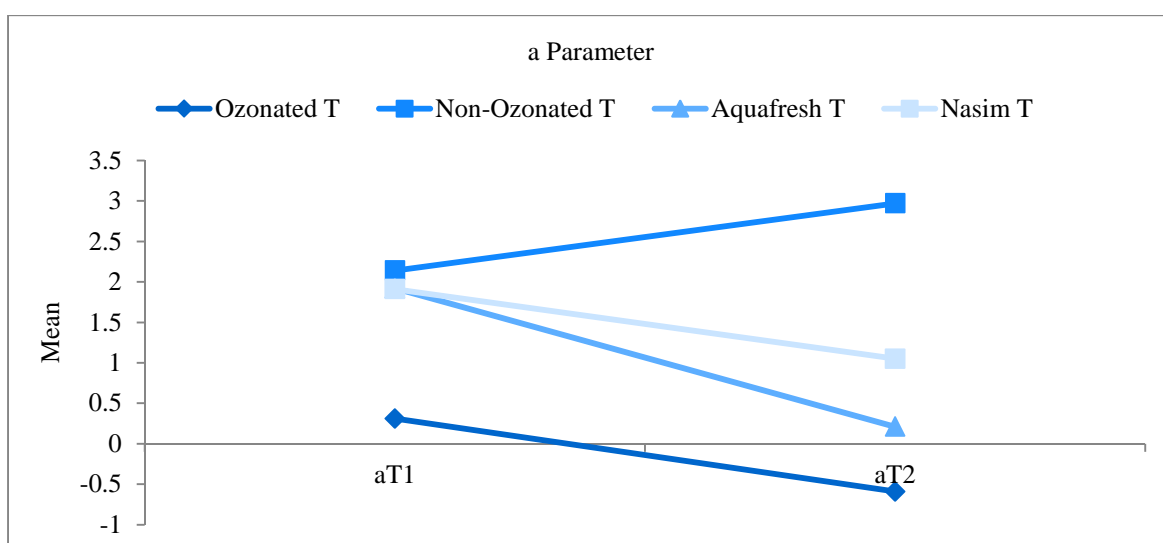


Figure 2. Mean of a^* parameter changes before and after brushing with different toothpastes

Among the four groups that were assessed by the colorimetric method, comparison was done between the color difference (ΔE) using the ANOVA test. Minimum color differences or color change was obvious in Group IV and between Stages I and II. The amount of color change among the two stages of color assessment among the experimental groups was not significant (Table 1).

The test result showed that there was a significant difference between the ranking of mean color change done by visual assessment among the four types of toothpastes ($P = 0.008$). Tukey post-hoc test indicated that there was a significant difference between

Groups I and III and with other groups regarding color changes by visual assessment (Table 2).

Discussion

Various bleaching agents have been introduced to whiten teeth, such as urea, nitric acid (or aqua fortis), chlorine, pyrozone, svpraksvl, hydrogen peroxide, ether, sodium perborate, carbamide peroxide, gly-oxide, proxigel,^{9,14,15} potassium cyanide, oxalic acid, ammonium, sodium peroxide, hydrogen dioxide¹⁶⁻¹⁸ and blue covarine.^{10,18,19} In addition, toothpastes containing carbamide peroxide 10% have also entered the marketed.²⁰

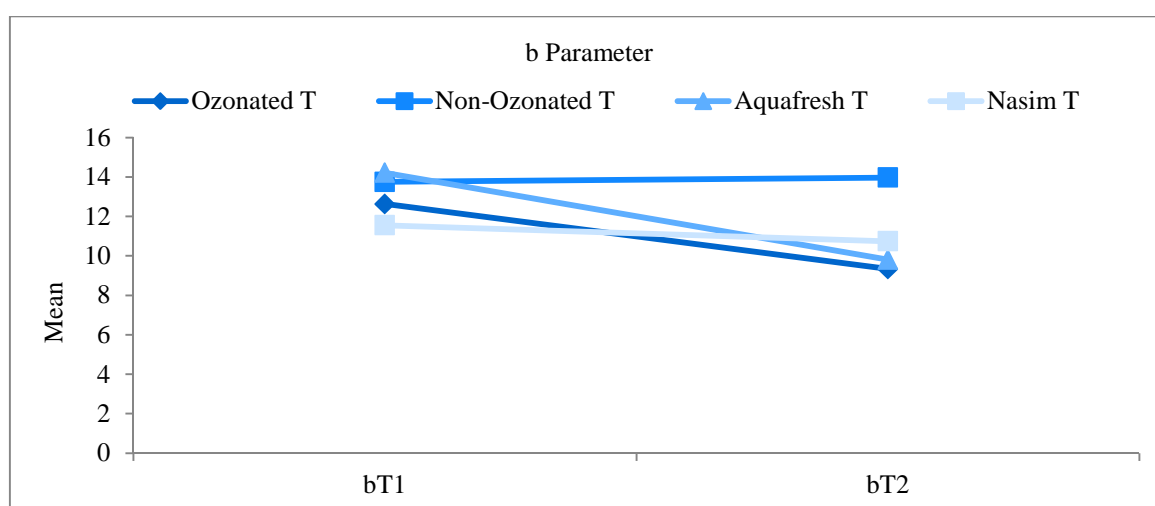


Figure 3. Mean of b* parameter changes before and after brushing with different toothpastes

Table 1. Comparison of mean color change with colorimetric assessment for the experimental toothpastes

Color change between 2 times	Experimental groups	N	Mean	SD	P
ΔE I, II	Ozonated T	12	7.64	2.48	0.073
	Non-ozonated T	12	6.41	3.46	
	Aquafresh T	12	7.81	4.26	
	Nasim T	12	4.57	2.60	

SD: Standard deviation

Table 2. Comparison of mean color change with the vita shade guide assessment for the experimental toothpastes

Color change between 2 times	Experimental groups	N	Mean Δ Vita (SD)
Δ Vita I, II	Ozonated T	12	-12.25 (1.48) ^a
	Non-ozonated T	12	-0.92 (1.78) ^b
	Aquafresh T	12	-5.00 (2.34) ^c
	Nasim T	12	-1.50 (1.16) ^b

Values with the same superscript letters are not statistically different; SD: Standard deviation

At present, the use of ozone in dentistry has been recommended for the sterilization of dental cavities, carious lesions, root canals, periodontal pouches and herpetic lesions.^{11,21} Medical grade ozone is a mixture of pure ozone and pure oxygen with the ratio of 0.05-5% ozone and 95.0-99.5% oxygen. Since ozone molecules are instable, ozone should be used immediately after being prepared. After preparation, half of the mixture begins to transform into ozone after an hour, whereas the other half becomes oxygen; therefore, making it impossible to store ozone for a long time. In order to control the breakdown of ozone to oxygen, a medium with aqueous properties to promote quick conversion or a medium with more viscous properties to retard conversion can be used.²¹⁻²³

The ozone used in this study was produced in Mashhad Pharmacology Research Laboratory by use of an American made ozone-generating machine with the ability to produce 13.5 g of ozone per hour. The ozone gas was blown into the olive oil tank by a pipe and with the speed of 13 g/h. The olive oil was converted to gel with completely oxidized properties after 48 h period. Ozonated olive oil with a pH of 8.5 was obtained by mixing with 1 m sodium hydroxide solution and then adding sodium lauryl sulfate to reach a 0.1% concentration. In this study, the ozone gas was used as a special gel for teeth whitening and its quality was compared with other toothpastes with have different components.

The evaluation impact of these materials to improve color was conducted by the colorimetric test and the measurement of sample colors were conducted based on the Lab coloring system and eye criteria or by ranking. In lab system, the color profile of the object is to be determined in three axis of value, hue and chroma; L* (lightness), a* (red-green) and b* (yellow-blue) respectively. To determine the color difference, the values listed can be calculated between two objects or two various phases and is shown by ΔE criteria.^{24,25}

In the current study, the color of teeth was determined for group classification and baseline color after staining with tea and the next step was to use the different toothpastes to compare their effects on whitening. In examining the color parameters of this study, the mean L* value increased significantly from baseline to after brushing, hence whiter teeth. Furthermore, a decrease in a* value is a sign of whitened teeth. The value of a* decreased in all toothpastes with the exception of non-ozone toothpastes.

The a* value reduction was significant in Aquafresh toothpaste and more noticeable in ozonated toothpaste groups. Regarding b* value, a decrease in b* value is also a sign of whitened teeth and this was observed in all toothpastes, except for the non-ozonated toothpaste. Obviously, the reduction of a* and b* values were related to the presence of whitening compounds of the ozonated and Aquafresh brand toothpastes just as in the bleaching agents.

In comparing the effect of using different toothpastes and the difference results, the changes of a* value was < b* and L* values, which was probably related to the properties of color staining material of the tea. Based on the L* a* b* color difference system, it is clinically important for the amount of $\Delta E = 3.3$, because this allows color change to be detectable by any observer. In evaluating the color changes in different times by the colorimetric method, ΔE was determined. We had the maximum mean color change in Aquafresh and ozonated toothpastes. All color changes were clinically visible and were above 3.3; however, these values were not statistically significant.

Thus, half of the null hypothesis of this study was accepted. In determining teeth color ranking by eye, the most mean color ranking changes were in the ozonated toothpaste and the least was in the non-ozonated one. There was a significant difference between mean color changes visually evaluated in the four types of

toothpaste. Therefore, the second half of the null hypothesis was rejected. It was determined that there was a significant difference between the color ranking changes between ozonated and aquafresh with other toothpastes. The average teeth color ranking change was brighter (12 and 5 score respectively) with the ozonated and aquafresh toothpastes; while, the amount of color lightening in the non-ozonated toothpaste and Nasim brand had almost the same color ranking; about one score. Similar to other studies that used the colorimetric and visual assessment methods, both methods showed visible clinical changes and the obtained data by both methods mostly confirmed each other. However, visual assessment is not as accurate as the colorimetric method.^{2,4,25}

Therefore, ozonated and aquafresh toothpastes caused tooth whitening after 6 weeks of brushing. Based on the obtain results, the role of ozone in the composition of the new toothpaste for bleaching of discoloration with a formulated ozone concentration were confirmed in this study. Ozone, such as the other dental bleaching materials that have already been introduced, is able to assist in removing tooth discoloration probably with the help of its oxidizing agent. Furthermore, the whitening role of ozonated toothpaste cannot be strictly attributed to surface wearing because in the non-ozonated toothpaste group the composition and the application regime was quite similar to ozonated toothpaste. Hence,

the ozone whitening role in this study was confirmed as in similar previous studies.^{12,25}

Considering the mentioned beneficial effects for ozone,^{11,26-29} it is recommended that in future studies the impact of the new ozonated toothpaste on other types of discolorations, other substrates and tooth-colored materials, persistence of its whitening effect, and changes on enamel microstructure in terms of tooth hardness level, wear and mineral content level be investigated. Also, by conducting a clinical trial, the long-term application of ozonated toothpaste, its biological effects and possible complications should be identified and resolved.

Conclusion

With the limitations of the study, it can be concluded that: The added benefit of ozone in toothpaste is that it has a bleaching effect on the teeth, and has some great results. Ozonated toothpaste caused significant whitening changes in discolored teeth in comparison to without ozonated toothpaste.

Conflict of Interests

Authors have no conflict of interest.

Acknowledgments

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