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A SURVEY OF TWO DENTAL BLEACHING SYSTEMS AND THEIR EFFECT ON NITRIC OXIDE AND CATALASE ENZYME IN THE GINGIVAL CREVICULAR FLUID

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ABSTRACT

OBJECTIVE: This study was carried out to examine the effect of two in-office bleaching systems by measuring the level of catalase enzyme (CAT) and nitric oxide (NO) in the gingival fluid (GCF) before and after bleaching. In addition, participants were asked to fill out an online survey to examine their satisfaction with the bleaching procedure.

MATERIALS AND METHODS: Thirty-six healthy young participants of 18- 25 years old were selected. They were divided into two groups according to the bleaching system used; Z1:Philips Zoom White of 25% H2O2 and Z2: Fläsh White Smile of 32% H2O2. Three sessions, 15 minutes each, were performed during the same visit for each participant. GCF samples were collected using a sterile periopaper before and after the bleaching session. A survey link was sent to all participants to examine their satisfaction.

RESULTS: There was a statistically significant increase in CAT and NO in the GCF of the Z2 when compared to the Z1. The bleaching survey revealed no statistically significant satisfactory experience 93.8% and 94.1% respectively regarding Z1 and Z2. There was no statistically significant difference between the Z2 and Z1 in all participants' answers except in the degree of gingival pain. The number of participants who reported no or mild pain in the Z2 was greater than those in the Zoom.

CONCLUSION: The higher percentage of H2O2 in Z2 increased CAT and NO release in the GCF. Participants in both groups were equally satisfied. Dental materials risks must be evaluated to prevent endangering human health.

KEYWORDS: Bleaching safety- Bleaching survey- H2O2 inflammatory mediators- Gingival crevicular fluid inflammatory markers

RUNNING TITLE: Bleaching Survey on Two Bleaching Systems and Their Effect on the Gingival Crevicular Fluid

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INTRODUCTION

The extensive rise of social media pressure and the unraveling competition of sharing self-images has increased the demand for white bright teeth. In-office bleaching is still the fastest and most effective method of obtaining attractive teeth in one visit. It is usually carried out with hydrogen peroxide H2O2 (HP) of 25- 38% concentration which is activated chemically and/or with heat or light. Activation of HPleads to the release of oxygen-derived free radicals which have strong oxidant capacities that allows them to diffuse into the enamel and dentine and break the double bonds of chromophore compounds into single bonds of smaller size and different configuration.(1) This in turn changes the optical properties of teeth structure, providing them with much lighter shades.(2)

The primary concern about tooth bleaching safety originates from the known toxicity of H2O2, because it is one of the reactive oxygen species (ROS) due to its ability to release free radicals including hydroxyl radicals. These free radicals cause oxidation reactions, leading to cell damage and many oral diseases such as cancer and other medical conditions (3). Fortunately, the human body has a variety of antioxidant defense mechanisms (non-enzymatic and enzymatic antioxidants) at tissue and cellular levels to protect cells from possible H2O2 injury and to restore the harm produced (4). Catalase CAT is an antioxidant intracellular enzyme found in human bodily fluids and tissues that converts hydrogen peroxide to oxygen and water, thereby protecting cells and tissues from oxygen-derived free radicals (4).

Salivary glands and their products secrete nitric oxide NO, an important inflammatory mediator (5). Catalase and Nitric oxide have been implemented in the current study as inflammatory biomarkers.

The objective of the current study was to investigate the implications of two in-office bleaching systems by measuring the levels of catalase enzyme and nitric oxide in the gingival crevicular fluid GCF before and after the bleaching session. In addition, each participant was asked to fill out an online survey to examine their satisfaction with the bleaching procedure.

The null hypothesis assumes that:

1. Bleaching with any of the tested bleaching agents will not affect the level of CAT and NO in the GCF.
2. There will be no difference between the two bleaching agents in the level of CAT and NO in the GCF after bleaching.
3. The survey results will not reveal any difference in the participants' answers after using any of the tested bleaching agents.
4. Subjects, Materials and Methods:
5. i. Participants criteria:

The Faculty of Dentistry, MSA University Ethical Committee first authorized this study protocol (identity ETH29). Then an announcement was made that a free bleaching session would be carried out for participants from (18-25) years old. Volunteers were examined and thirty- six of those who met the required criteria were selected. After knowing about the procedure's steps and potential hazards, all participants signed an informed consent. Inclusion criteria were; having their maxillary and mandibular anterior teeth free from caries, non-carious lesions and were vital. All participants had to be eager to participate in the study and of good oral hygiene and. Exclusion criteria included; smoking, gingival recession, teeth sensitivity, soft tissue lesions, malocclusion, allergy to the bleaching agent, systemic illness, pregnant and nursing women. The examination and bleaching sessions were carried out at the postgraduate clinic of the Faculty of Dentistry, MSA University. Participants were randomly divided into two groups of eighteen:

Group1(Z1): Philips Zoom White Speed (P Zoom WS) was used for them.

Group 2 (Z2): Fläsh White Smile (Fläsh WS) was used for them.

.2ii. Materials used:

Two chair-side light-activated bleaching systems were used in this study. The first one was P Zoom WS with Liquidam for gingival protection, Relief desensitizing agent containing amorphous calcium phosphate (ACP) and LED activation lamp of wavelength range from 400 to 505 nanometers and 190-50 mW/cm2 power (Discus Dental, LLC, Los Angeles, CA 90094, U.S.A (

P Zoom WScontains: Hydrogen peroxide 25%, glycerin, polypropylene glycol, water, polyethylene, Mentha piperita oil, eugenol, dihydrate. bis (D-gluconato-O1, O2). The second one was Fläsh WS with a specially formulated gingival protector, Fläsh after Whitening Mousse containing (30 % Xylitol, 4.2% Potassium Nitrate, 1450 ppm Sodium Fluoride, Sodium Phosphate, Calcium Nitrate, Sodium Saccharin, Natural Mentha Piperita, Poloxamer 338 and water) in addition to Fläsh WS Whitening Lamp GmbH of 460 nm wavelength and 190-50 mW/cm2 power (Weinheimer Str. 6, 69488 Birkenau, Germany)

Fläsh WScontains Hydrogen Peroxide 32%, chlorophyll, organic amines, and silicon dioxide .

The application of each bleaching system followed the guidelines provided by the manufacturer .

1. iii. Allocation concealment and random sequence generation:

The names of each bleaching system were concealed and assigned sequential numbers that denoted their sequence of use by a non-biased volunteer not involved in the study. Thirty-six cards prepared and these cards were given a number according to the bleaching system used. Each participant chose a card and recorded his/ her name on it for easy recording at the start of the bleaching session. They were not allowed to select or know the type of bleaching agent used for him/her(6).

.2iv. Patient preparation and sample collection:

Every participant was instructed to perform proper oral hygiene measures. Professional supra, sub-gingival scaling and polishing, was carried out to each participant a week prior to the bleaching session. They were permitted to only drink water and avoid any other food or drink one hour before the bleaching session. At the start of the bleaching session, teeth isolation was done using low-volume suction and cotton rolls to prevent sample contamination from saliva. The six anterior teeth of the upper and lower jaw were washed with water before being dried with gentle air stream directed perpendicular to the gingival border (7).Then for 30 seconds, sterile filter paper strips (Periopaper, Amityville, New York, U.S.A) were carefully placed in the gingival sulcus of the teeth to be bleached until the initial resistance was felt. Then the collected filter paper strips were stored in sterile Eppendorf containers (Eppendorf, Warszawa, Poland) labeled by the participant’s name, the number of his/her selected card, and the initials (B) before or (A) after the bleaching session.

.2v. Measurement of the gingival index:

Before and after the bleaching session the gingival index of the six anterior teeth of the upper and lower jaw was measured by running a Williams periodontal probe (LASCOD Zeffiro, Sesto Fiorentino, Florence, Italy) inside the buccal, palatal/lingual, mesial and distal gingival sulcus. The total score of each tooth was added to the score of the other teeth of the same participant and the average was calculated. The gingival index was

calculated according to Löe H and Silness J reference values(8(

.2vi. Bleaching material application:

Single-use lip balm (Safetec of America, Buffalo, New York, U.S.A) was used to protect the participant's corners of the mouth and lips. Isolation was done using the tongue and cheek retractor supplied with each kit in addition to the low-volume suction and cotton rolls that were placed in the labial and buccal vestibule. The gingival barrier of each bleaching system was applied on the free and attached gingiva of the teeth to be bleached and then light activated for one second using the light emitting diode LED (Elipar Deep Cure-S, 3M ESPE, St. Paul, Minnesota, USA). Both the operator and participants wore LED protective eyewear. Each bleaching agent was auto-mixed as directed by the manufacturer and applied in a two mm thick layer on the labial surfaces of the teeth to be bleached. The first 15 minutes of each bleaching session, the LED activation system was set to the maximum power of 190 mW/cm2; then the next 15 minutes were set to the medium power of 120 mW/cm2 for two sessions this was done for both bleaching systems. The three sessions lasted in total of 45 minutes. After the last session and removing any remnants of the bleaching agent, the gingiva and soft tissue were evaluated for any evidence of inflammation. The GCF was collected again using the periopaper as explained before. Desensitizing agent of each bleaching system was then applied to the bleached teeth and left for ten minutes. Participants were instructed to abstain from drinking and eating for 30 minutes, to keep their teeth clean by brushing and flossing after each meal, to avoid colored beverages and food for two weeks.

2. vii. Measurement of catalase and nitric oxide:

All GCF samples were kept at -40°C until laboratory analyses. The day before the analyses GCF samples were kept at +4°C overnight on a shaking platform to allow GCF elution from the strips. Before processing and analysis, each sample was vortexed for one minute. Total NO is calculated by conversion of nitrate to nitrite using nitrate reductase enzyme. The level of NO was calculated by determining the total nitrite and nitrate concentrations in the sample using the Green et al.,1982 method (9). After the reaction, nitrite was colorimetrically detected as an azo dye product of the Griess reaction.

The Griess reaction is based on a two-step diazotization reaction in which acidified nitrite creates a nitrosating agent that reacts with sulfanilic acid to form a diazonium ion. This ion then combines with N-(1-naphthyl)-ethylenediamine dihydrochloride (NEDD) to form a red chromophore azo derivative at 540–570 nm.

Measurement of catalase enzyme was carried out according to the method of Aebi(10) where a calculated quantity of (HP) react with the collected GCF samples to determine the activity of CAT. A catalase inhibitor was added to stop the reaction after exactly one minute. In the presence of peroxidase (HRP), remaining (HP) reacts with 3,5-Dichloro-2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the activity of CAT in the original sample. The NO level was calculated as (μmol/L), while CAT level was calculated as U/L both were calculated from the collected participant's GCF before and after the bleaching session.

2.viii. Filling out an online questionnaire:

A survey link was prepared using Survey Monkey software (SurveyMonkey 2020, One Curiosity Way, San Mateo, California, USA, 94403) and was sent to all participants to examine their satisfaction, feedback, and the efficiency of the bleaching agent.

2- ix. Statistical analysis:

The data was analyzed using IBM SPSS software program version 20.0. (IBM Corporation, Armonk, New York, USA). Data analysis of nitric oxide and catalase enzyme was performed by Kolmogorov-Smirnov test to ensure that the distribution of variables was normal. In order to compare the two sets of normally distributed quantitative data, the student t-test was used. While, Mann Whitney test was employed to compare between the two groups for non-normally distributed quantitative variables and the Wilcoxon signed ranks test was used to compare the two periods of non-normally distributed quantitative variables. As for normally distributed quantitative data, the paired t-test was used to compare the two periods. The significance of the obtained results was determined at a 5% level**.** As for the questionnaire analysis qualitative data were described using number and percent. Chi-square test was used for categorical variables, to compare between the different groups. Fisher's Exact or Monte Carlo tests were used as correction for Chi-square when more than 20% of the cells have expected count less than five. The significance of the obtained results was determined at a 5% level**.**

RESULTS

The gingival index was measured before and after the bleaching session. The gingival index of all participants recorded a zero value (Healthy gingivas) before and after the bleaching session. That is why no statistical analysis was performed for this measurement.

Table (1) and Figures (1, 2) show the comparison between P Zoom WS (Z1) and Fläsh WS (Z2) bleaching systems according to CAT and NO level. The analysis of the results revealed that there was no statistically significant difference in the CAT and NO level between both groups before bleaching. After bleaching, both CAT and NO levels increased significantly in Z2 vs Z1 where p= 0.012 and p<0.001, respectively. In Z1 there was no statistically significant difference in the CAT and NO level before and after bleaching. In Z2, CAT

level did not statistically significantly increase after bleaching and accordingly, at the end of the study, there was no statistically significant difference in the increase in CAT before and after bleaching between both groups p= 0.161. While the NO level statistically significantly increased after bleaching in Z2 p<0.001. At the end of the study, there was a statistically significant difference in the increase in NO before and after bleaching between both groups with the Z2 showing a statistically significant higher increase p<0.001.

Table (1): Comparison between P Zoom WS and Fläsh WS bleaching systems according to CAT and NO

Variable	Study Period	Mean/Median	P Zoom WS (n = 18)	Fläsh WS (n = 18)	Test of sig.	p
Catalase	**Before x10^{-3}**	Mean ± SD.	1.6 ± 0.5	1.4 ± 0.4	U= 136	0.424
		Median (Min. – Max.)	1.5 (0.9 – 3.4)	1.5 (0.3 – 1.9)		
	After x10^{-3}	Mean ± SD.	1.6 ± 0.4	1.7 ± 2.1	U= 83.0*	0.012*
		Median (Min. – Max.)	1.5 (1.1 – 2.8)	0.8 (0.2 – 6.9)		
		P0	0.663	0.214		
	Increase x10^{-3}	Mean ± SD.	-0.1 ± 0.4	0.3 ± 2	U=117.0	0.161
		Median (Min. – Max.)	0 (-0.7 – 0.7)	-0.5 (-1.3 – 5.3)		
Nitric Oxide	**Before**	Mean ± SD.	3 ± 1.3	2.7 ± 1.2	t=0.623	0.537
		Median (Min. – Max.)	3 (0.5 – 5.5)	2.6 (1.1 – 5.3)		
	After	Mean ± SD.	3.6 ± 1.7	15.5 ± 4.2	t=11.275*	<0.001*
		Median (Min. – Max.)	3.5 (0.5 – 7.4)	15.8 (8.7 – 22.4)		
		p0	0.282	<0.001*		
	Increase	Mean ± SD.	0.7 ± 2.5	12.8 ± 4.4	U=3.0*	<0.001*
		Median (Min. – Max.)	0.9 (-3.5 – 6.9)	13.4 (3.4 – 19.8)		

p: p value for comparing between P Zoom WS and Fläsh WS

p_0: p value for Paired t-test for comparing between before and after in each device

*: Statistically significant at $p \leq 0.05$

Regarding the questionnaire results, Table (2), Figure (3) show the comparison between Z1 and Z2 bleaching systems according to bleaching satisfaction survey results. Statistical analysis revealed that there was no statistically significant difference in the degree of satisfaction in both the Z1
and Z2 where the percentage of satisfaction was 93.8% and 94.1 % respectively.

Table (2): Comparison between P Zoom WS and Fläsh WS bleaching systems according to bleaching satisfaction survey

Q	Bleaching satisfaction survey	P Zoom WS (n = 16)		Fläsh WS (n = 17)		χ^2	p
		No.	%	No.	%		
1	**How was your bleaching experience?**						
	Satisfied	**15**	**93.8**	**16**	94.1	0.002	^{FE}p= 1.000
	Not satisfied	1	6.3	1	5.9		
2	**After bleaching did you experience any gingival inflammation?**	**(n=15)***		**(n=16)***			
	No	2	13.3	2	12.5	0.005	^{FE}p= 1.000
	Yes	13	86.7	14	87.5		
3	**After bleaching did you experience any allergy?**						
	No	10	62.5	12	70.6	0.243	0.622
	Yes	6	37.5	5	29.4		
4	**After bleaching did you experience any change in the taste sensation, even if temporary?**						
	No	16	100.0	17	100.0	–	–
	Yes	0	0.0	0	0.0		
5	**Did you feel any pain after bleaching?**						
	No	8	50.0	8	47.1	0.029	0.866
	Yes	8	50.0	9	52.9		
6	**Was this pain related to the gingiva or the teeth?**						
	Teeth	8	50.0	8	47.1		
	Gingiva	1	6.3	1	5.9	0.326	^{MC}p= 1.000
	None	7	43.8	8	47.1		
7	**Degree of the gingival pain**						
	No pain	9	56.3	7	41.2		
	Mild	1	6.3	7	41.2	10.431*	^{MC}p= 0.007*
	Moderate	5	31.3	0	0.0		
	Severe	1	6.3	3	17.6		
8	**Did you brush your teeth before the bleaching session?**						
	No	4	25.0	0	0.0	4.836*	^{FE}p= 0.044*
	Yes	12	75.0	17	100.0		
9	**Did you brush your teeth after the bleaching session?**						
	No	9	56.3	8	47.1	0.279	0.598
	Yes	7	43.8	9	52.9		
10	**After the bleaching session, did you experience gingival bleeding during brushing?**						
	No	16	100.0	17	100.0	–	–
	Yes	0	0.0	0	0.0		

χ^2: **Chi square test** MC: **Monte Carlo**
FE**: Fisher Exact**
*: Statistically significant at $p \leq 0.05$ * 1 case skipped from the sample

Also, there was no statistically significant difference between both groups in all participants answers except in the degree of gingival pain where there was no or mild pain reported in 82.4 % of Z2 and 62.6 % of Z1 participants where p=0.007. In addition, there was a statistically significant difference in the number of participants who brushed their teeth before the bleaching session; 100% of Z2 and 75% of Z1 participants p=0.044.
In both groups, there was no change in the taste sensation and the gingival inflammation was recorded in almost 87% (Z1: 86.7and Z2: 87.5) and it was not statistically significant when comparing both groups. Half of the participants recorded pain, this pain was reported to be from the teeth in 47% and 50 % in the Z2 and Z1 respectively. There was no statistically significant difference in teeth brushing after the bleaching session where it decreased to be 52.9% in the Z2 and 43.8 % in the Z1, but no one experienced gingival bleeding during brushing.√ √√

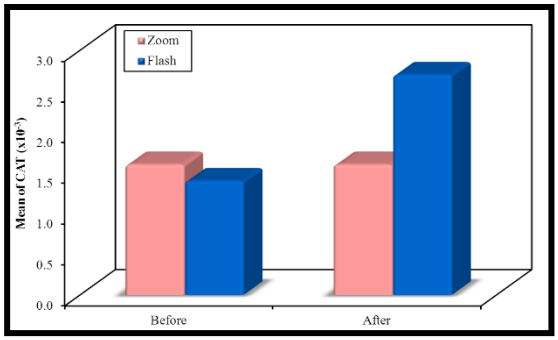

Figure (1): Comparison between P Zoom WS and Fläsh WS bleaching systems according to CAT enzyme

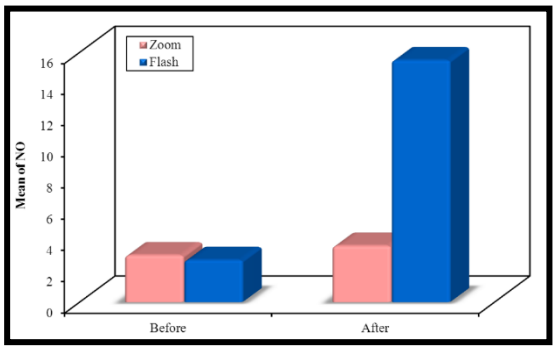

Figure (2): Comparison between P Zoom WS and Fläsh WS bleaching systems according to NO

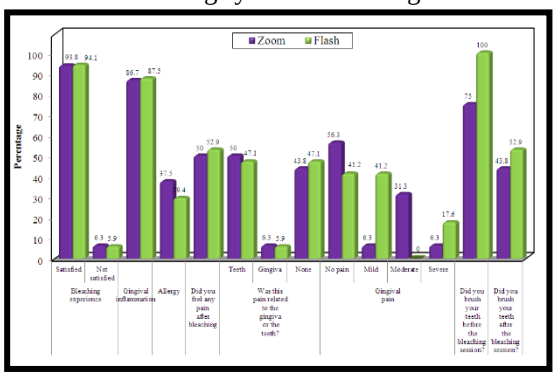

Figure (3): Comparison between P Zoom WS and Fläsh WS bleaching systems according to bleaching satisfaction survey

DISCUSSION

Dental bleaching agents depend on reactive oxygen species (ROS) to oxidize teeth stains and the heat produced by the light source in power bleaching techniques enhance the reaction by increasing (HP) decomposition and increase its penetration.(11) The safety of these considerably high concentration bleaching agents have to be well studied to avoid any adverse effect in the human body. In addition to scientific concerns, the impact of these free radicals on oral tissues led to legal concerns about their usage in dentistry.(12) The level of risk of these agents is affected by the bleaching gel composition, the used technique, and the patient's response to the bleaching procedure.(13) Several studies have been shown that hydrogen peroxide and its sub-products can easily diffuse through the dentinal tubules to the pulp tissue or the inflamed periodontium reducing cell viability, causing degradation of extracellular-matrix and all that may lead to partial or total pulp necrosis.(12,13) Oxidative stress reactions occur when an imbalance occurs between the body's oxidative and antioxidant capacity leading to oxidation, which leads to several diseases.(14) The most damaging radicals in the body include hydroxyl radicals, hydrogen peroxide, oxygen singlet, superoxide and anion free radicals.(15) These free radicals are highly reactive and if not controlled may damage proteins, DNA, lipids and carbohydrates in the nucleus and cell membranes. (16) The defense mechanism of the body takes place to stop lipid peroxidation and DNA transformation, which results in cell lysis and death.(17)

Several enzymes exist in the body fluids and tissues, including saliva and GCF, these enzymes effectively metabolize H_2O_2.(18) Catalase is considered an endogenous antioxidant and a key enzyme that is capable of rapid breakdown of reactive oxygen species that are present in hydrogen peroxide into water and oxygen reducing its harmful effects.(19) Nitric oxide is synthesized by NO synthase enzyme, which is responsible for the release of NO for physiological purposes and the other is induced by cytokines as part of the immunological response.(20) The level of redox imbalance can be measured by detecting these inflammatory biomarkers as NO and CAT (21) using either chromatographic, spectrophotometric or electrochemical methods. (22) Saliva(23) and/ or GCF(24) component collection is a straightforward and non-invasive method for detecting these markers. That is why in this study, the safety of two in- office bleaching agents was carried out by measuring the amount of CAT and NO present in the GCF before and after the bleaching session.

The results of the present study showed no change in CAT levels before and after the application of the P Zoom WS bleaching agent, but there was a statistically significant increase in CAT level after the application of Fläsh WS bleaching agent indicating the occurrence of inflammation after bleaching with Fläsh WS. This was explained from previous studies who demonstrated that higher concentration and duration of the bleaching agent application on the enamel, the greater the (HP) penetration to the pulp chamber and the more severe the unfavorable effects on pulp cell.(25) Although studies showing the association of CAT with periodontal disease are limited, the inflammatory effect of Fläsh WS bleaching agent in the form of elevation of CAT level is similar to that obtained by Panjamurthy et al., 2005(21) and Garg et al., 2006(26). Borges et al., 2007(27) who did not find any statistically significant difference. On the other hand, Tonguc et al., 2011(28) and Trivedi et al.,

2015(17) found a significant decrease in CAT level in GCF of patients with periodontitis.

Regarding NO level, P Zoom WS caused an insignificant increase in its level whereas Fläsh WS bleaching agent showed a statistically significant increase in NO level. In literature many studies have identified the alterations in the NO amounts. Some studies (29), (30) found insignificant increase of NO, while others (31) found that there was a statistically significant increase in NO level in GCF of the diseased sites when compared to the healthy sites. Hirose et al. 2001 (32) explained that higher levels of NO were associated with the production of NO by macrophages and polymorphonuclear leukocytes inside the isoform NO synthase cycle, which contributes to the inflammatory process. (32) Although Aurer et al., 2001(33) found that NO level was reduced in *the saliva* of patients who had periodontitis, Topcu et al., 2014 (16) explained that quantitative and qualitative evaluation of *gingival* crevicular fluid have a better diagnostic value than saliva.

An online questionnaire using Survey Monkey software was carried out to detect the participants' satisfaction, opinion, the presence of any side effects and/ or problems associated with the bleaching procedure. The bleaching survey revealed no statistically significant satisfactory experience 93.8% and 94.1% respectively regarding zoom and flash bleaching systems.

The results of the questionnaire revealed allergic reactions in 29.4 % of the Z2 and 37.5 % of the Z1. This agrees with Watt et al. 2004 (34) who found that bleaching agents containing 10% (w/w) carbamide peroxide exhibited acute and sub-acute cytotoxic effects at levels more than 5 g/kg/day. This is equivalent to 0.3 to 1.8 mg/kg (body weight)/day H_2O_2. Both groups revealed the absence of taste sensation loss after the bleaching session. Although other studies showed that patients reported metallic taste sensation immediately after bleaching; which usually disappears after few hours.(35) The participants revealed that the gingival inflammation increased to be 87% after bleaching, but there was no gingival bleeding. The absence of gingival bleeding agrees with Sato et al., 2013(36) who found that bleaching did not affect cysteine cathepsin or matrix metalloproteinase activities in the examined GCF indicating that the procedure did not cause gingival damage. This agrees with this study results regarding the gingival index which recorded zero (healthy gingiva) before and just after bleaching. Although Zouair et al., 2012(37) found that bleaching agents of high (HP) concentration did not only affect the gingival epithelium but also extended to the subepithelial tissues.

There was no statistically significant difference between Z2 and Z1 in all participants answers except in the degree of gingival pain where 82.4 % and 62.6 % of the participants reported no or mild pain, in Z2 and Z1 respectively. This agrees with Freedman and Greenwall 2001(35) who reported that after bleaching gingival pain occurs as a result of gingival ulceration and burns that appear as white lesions followed by a red rim and that usually disappear after a few minutes and do not cause permanent damage. The presence of proper gingival seal and protection of the oral tissues during the bleaching procedure prevents the occurrence of these lesions. In this study strict gingival and soft tissue isolation was carried out. Both groups recorded a high percentage of gingival inflammation but there was no statistically significant difference between both groups this agrees with Jorgensen and Carroll 2002.(38)

It worth mentioning that only one patient in each group reported that the source of pain was from the gingiva. That is why a question was added to the questionnaire to examine the participants' answers and to make sure whether they did not have gingival pain or the gingival pain was masked by the dental pain. There answers revealed that the gingival pain was mostly mild to moderate.

All participants were instructed to perform proper oral hygiene measures before the bleaching session. The results of the questionnaire revealed that there was a statistically significant difference in the number of participants who brushed their teeth before the bleaching session; 100% of Z2 and 75% of Z1 participants p=0.044. After the bleaching session participants were instructed to keep their teeth clean by brushing and flossing after each meal. The results of the questionnaire revealed that there was no statistically significant difference in teeth brushing after the bleaching session where it decreased to be 52.9% in the Z2 and 43.8 % in the Z1. The reason for the decrease in the brushing percentage after bleaching may be due to the presence of pain after the bleaching session which agrees with their answers regarding the source of pain where 47% and 50 % in the Fläsh WS and P Zoom WS participants respectively recorded pain from their teeth. This agrees with several studies (6), (39), (25) who reported that the highest penetration of (HP) and its subproducts to the pulp chamber occurred when 35% (HP) bleaching agent was used in one session 3x15 minutes and the most intense pulp reaction was observed. This lead to pulp cells affection with the release of inflammatory mediators (40), stimulation of the sensory nerves (41) with partial necrosis in the pulp connective tissue.(42) It worth mentioning that it was observed in this study that Fläsh WS bleaching gel is less viscous than that of P Zoom WS and this may facilitate it's accidental flow to the gingival tissues. That is why extra care should be done to protect the gingival margin.

As a consequence, every product has its drawback, and there is no treatment without risks. That is why risks have to be properly evaluated and examined to prevent endangering human health. Following manufacturer's instructions and preventing the abuse and misuse of oral products will definitely minimize their risks.

CONCLUSIONS

Fläsh WS bleaching agents increased the level of nitric oxide and catalase enzyme in the gingival crevicular fluid. Patient were equally satisfied with both bleaching agents. The degree of gingival pain was greater in the Fläsh WS, although the degree of gingival inflammation was almost the same in both groups. In both groups, there was no change in taste sensation and no one experienced gingival bleeding during brushing.

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REFERENCES

1. Dahlstrom SW, Heithersay GS, Bridges TE. Hydroxyl radical activity in thermo-catalytically bleached root-filled teeth. Endodontics & dental traumatology. 1997;13:119–25.
2. Alqahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dental Journal. 2014;26:33–46.
3. Baltacioğlu E, Akalin FA, Alver A, Değer O KE. Protein carbonyl levels in serum and gingival crevicular fluid in patients with chronic periodontitis. Archives Of Oral Biology. 2008;53:716–22.
4. Diab-Ladki R, Pellat B, Chahine R. Decrease in the total antioxidant activity of saliva in patients with periodontal diseases. Clinical oral investigations. 2003;7:103–7.
5. Looms D, Tritsaris K, Pedersen AM, Nauntofte B, Dissing S. Nitric oxide signalling in salivary glands. Journal of Oral Pathology and Medicine. 2002;31:569–84.
6. El Wakeel A, Fadel M, El Baz M. Clinical Evaluation of Two Different Bleaching Systems (Flash and Zoom) on Post Bleaching Hypersensitivity (a Randomized Clinical Trial). Egyptian Dental Journal. 2018;64:1673–80.
7. Griffiths GS. Formation, collection and significance of gingival crevice fluid. Periodontology 2000. 2003;31:32–42.
8. Löe H, Silness J. Periodontal Disease In Pregnancy. I. Prevalence And Severity. Acta odontologica Scandinavica. 1963;21:533–51.
9. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Analytical biochemistry. 1982;126:131–8.
10. Aebi H. Catalase in vitro. Methods in enzymology. 1984;105:121–6.
11. De Moor RJG, Verheyen J, Diachuk A, Verheyen P, Meire MA, De Coster PJ, et al. Insight in the Chemistry of Laser-Activated Dental Bleaching. The Scientific World Journal. 2015; 2015: 650492.
12. Qi F, Huang H, Wang M, Rong W WJ. Citation: Applications of Antioxidants in Dental Procedures. 2022;11:2492
13. Carey CM. Tooth whitening: what we now know. The journal of evidence-based dental practice. 2014;14 Suppl(SUPPL.):70–6.
14. Soares DG, Ribeiro APD, da Silveira Vargas F, Hebling J, de Souza Costa CA. Efficacy and cytotoxicity of a bleaching gel after short application times on dental enamel. Clinical Oral Investigations. 2013;17:1901–9.
15. Carnelio S, Khan SA, Rodrigues G. Definite, probable or dubious: Antioxidants trilogy in clinical dentistry. British Dental Journal. 2008;204:29–32.
16. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 2010;4:118- 126.
17. Çakir EG, Özcan S, Tulunoglu I, Üçtaşli MB, Tulunoglu O. Efficacy of In-office Bleaching on Microhardness of Permanent Teeth with Antioxidant Re-hardening. The Open Dentistry Journal. 2019, 13, 436-442.
18. Trivedi S, Lal N, Mahdi A, Singh B, Pandey S. Association of salivary lipid peroxidation levels, antioxidant enzymes, and chronic periodontitis. The International journal of periodontics & restorative dentistry. 2015;35:e14–9.
19. Toczewska J, Maciejczyk M, Konopka T, Zalewska A. Total Oxidant and Antioxidant Capacity of Gingival Crevicular Fluid and Saliva in Patients with Periodontitis: Review and Clinical Study. Antioxidants (Basel). 2020;9:450
20. Ahn J, Nowell S, McCann SE, Yu J, Carter L, Lang NP, et al. Associations between catalase phenotype and genotype: modification by epidemiologic factors. Cancer epidemiology, biomarkers & prevention. 2006;15:1217–22.
21. Moncada S, Higgs EA. Endogenous nitric oxide: Physiology, pathology and clinical relevance. European Journal of Clinical Investigation. 1991;21:361–74.
22. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell and Molecular Biology Letters. 2005;10:255-264.
23. Koss-Mikołajczyk I, Baranowska M, Namieśnik J, Bartoszek A. Determination of antioxidantactivity of phytochemicals in cellular models by fluorescence/luminescence methods. 2017;71:602–17.
24. Parwani SR, Chitnis PJ, Parwani RN. Salivary nitric oxide levels in inflammatory periodontal disease - a case-control and interventional study. International journal of dental hygiene. 2012;10:67–73.
25. Bibi T, Khurshid Z, Rehman A, Imran E, Srivastava KC, Shrivastava D. Gingival Crevicular Fluid (GCF): A Diagnostic Tool for the Detection of Periodontal Health and Diseases. Molecules. 2021;26(5).

26. Garg N, Singh R, Dixit J, Jain A, Tewari V. Levels of lipid peroxides and antioxidants in smokers and nonsmokers. Journal of periodontal research. 2006 Oct [cited 2023 Sep 9];41(5):405–10. Available from: https://pubmed.ncbi.nlm.nih.gov/16953817/
27. Borges I Jr, Moreira EA, Filho DW, de Oliveira TB, da Silva MB, Fröde TS. Proinflammatory and oxidative stress markers in patients with periodontal disease. *Mediators Inflamm*. 2007;2007:45794.
28. Tonguç MÖ, Öztürk Ö, Sütçü R, Ceyhan BM, Kılınç G, Sönmez Y, et al. The impact of smoking status on antioxidant enzyme activity and malondialdehyde levels in chronic periodontitis. Journal of periodontology. 2011;82:1320–8.
29. Menaka KB, Ramesh A, Thomas B, Kumari NS. Estimation of nitric oxide as an inflammatory marker in periodontitis. Journal of Indian Society of Periodontology. 2009;13(2):75.
30. Thomas B, Kumari S, Ramesh A, Rao A, Inasu S. Evaluation of serum and salivary sialic acid and nitric oxide levels in chronic periodontitis patients. International Journal of Applied Dental Sciences. 2016;2:74–6.
31. Tözüm TF, Turkyilmaz I, Yamalik N, Karabulut E, Türkyilmaz AS, Eratalay K. Analysis of the possibility of the relationship between various implant-related measures: An 18-month follow-up study. Journal of Oral Rehabilitation. 2008;35:95–104.
32. Hirose M, Ishihara K, Saito A, Nakagawa T, Yamada S, Okuda K. Expression of cytokines and inducible nitric oxide synthase in inflamed gingival tissue. Journal of periodontology. 2001;72:590–7.
33. Aurer A, Aleksić J, Ivić-Kardum M, Aurer J, Čulo F. Nitric Oxide synthesis is decreased in periodontitis. Journal of Clinical Periodontology. 2001;28:565–8.
34. Watt BE, Proudfoot AT, Vale JA. Hydrogen peroxide poisoning. Toxicological reviews. 2004;23:51–7.
33. Aurer A, Aleksić J, Ivić-Kardum M, Aurer J, Čulo F. Nitric Oxide synthesis is decreased in periodontitis. Journal of Clinical Periodontology. 2001;28:565–8.
34. Watt BE, Proudfoot AT, Vale JA. Hydrogen peroxide poisoning. Toxicological reviews. 2004;23:51–7.
35. Freedman G A, Greenwall L. Bleaching Techniques in Restorative Dentistry. Bleaching Techniques in Restorative Dentistry:an illustrated guide. Chapter: 7 Power Bleaching and In-office Techniques. Martin Dunitz, London, United Kingdom, eBook, ©2001
36. Sato C, Rodrigues FA, Garcia DM, Vidal CMP, Pashley DH, Tjäderhane L, et al. Tooth bleaching increases dentinal protease activity. Journal of Dental Research. 2013;92:187–92.
37. Attia-Zouair MG, Adawy HA, Fekry Khedr MM. Ultrastructure of the cellular response of rabbits' gingivae to the adverse effects of light enhanced bleaching. Life Science Journal. 2012;9:910–23.
38. Jorgensen MC, Carroll WB. Incidence of tooth sensitivity after home whitening treatment. Journal of the American Dental Association (1939);133(8):1076–82.
39. Camargo S, Valera M, Camargo C, Gasparotomancini M, Menezes M. Penetration of 38% Hydrogen Peroxide into the Pulp Chamber in Bovine and Human Teeth Submitted to Office Bleach Technique. Journal of Endodontics. 2007;33:1074–7.
40. Dias Ribeiro AP, Sacono NT, Lessa FCR, Nogueira I, Coldebella CR, Hebling J, et al. Cytotoxic effect of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells. Oral surgery, oral medicine, oral pathology, oral radiology, and endodontics. 2009;108:458–64.
41. Cecarini V, Gee J, Fioretti E, Amici M, Angeletti M, Eleuteri AM, et al. Protein oxidation and cellular homeostasis: Emphasis on metabolism. Biochimica et biophysica acta. 2007;1773:93–104.
42. Soares DG, Marcomini N, Basso FG, Pansani TN, Hebling J, de Souza Costa CA. Indirect cytocompatibility of a low-concentration hydrogen peroxide bleaching gel to odontoblast-like cells. International endodontic journal. 2016;49:26–36.