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EFFECT OF DIFFERENT ATTEMPTS OF BLEACHOREXIA ON THE MINERAL CONTENT OF TOOTH STRUCTURE

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ABSTRACT

OBJECTIVE: In vitro evaluation of the effect of bleachorexia on mineral content of tooth structure. **Materials and methods:** All labial surfaces of 30 sound human premolars were protected with nail varnish except a window of labial enamel. Group I: Deluxe home whitening kit was applied 1 hour once daily for 14 days, rinsed by distilled water and stored in artificial saliva. Minerals were measured using EDX, remineralization with NovaMin® for 10 minutes. Group II: As in gp I but abuse of bleaching 2 hours daily for 7 successive days (14 cycles). Group III: Bleaching for an hour twice daily for 3 days and 1 hour in the fourth day (seven cycles), remineralization and all procedures were repeated for 7 more cycles. **Results:** All data was collected and statistically analyzed. Crest was used to compare the mineral content in the 3 tested gps at 95% level of significance. Fluoride content was affected significantly by the bleaching in the 3 tested gps. After remineralization Gp I&II were affected (p values 0.043 &0.00) whi le Gp III didn't gain any benefit (p value of 0.160). Comparing the bleaching cycles vs remineralization for 10 min all gps recorded a significant difference with p values of 0.011, 0.059 & 0.033 for gps I, II&III respectively. Phosphate content in all gps were significantly affected by bleaching & remineralization (p values of 0.001). Using Tukey test, the time of application of bleaching has an oblivious effect on the phosphate and Ca content which couldn't regain it by remineralization.

KEYWORDS: Bleachorexia, minerals content, enamel

INTRODUCTION

Bleaching became a popular procedure with patients who are seeking improvements in the appearance of their discolored teeth. Tooth discoloration is a common complaint in most of the dental clinics and tooth bleaching has been considered the preferred esthetic treatment alternative, being more conservative, safe and with predictable results [(1)]. Tooth whitening is achieved by the chromophore theory, whereby the organic chromophores in the tooth structure are attacked by HP. The organic chromophores have electron rich areas which slowly interact with the reactive

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O_2 species through an oxidation-reduction reaction (the redox reaction) [2], this reaction alters the optical properties of the chromophore chains converting them into simpler & lighter chemical intermediates, thus eliminating the stained appearance [3].

The search for whiter teeth pattern has encouraged the consumption of bleaching agents. Due to this demand, a high diversity of products has been launched, with a portion of these characterized by low-cost sale without a dentist office visit [4].

Some patients may have unhealthy obsession towards teeth whitening known as bleachorexia. This leads to the abuse of the bleaching agents without respecting the manufacturer's instructions. The abuse might be through over bleaching which can be done by either using too much gel, leaving the product on the teeth for too long or frequent use of the product (1), which can lead to loosing of mineral content which in turn decreases microhardness, thus increases enamel susceptibility to demineralization [5]. Thus, remineralizing treatment received a lot of attention by both clinician and researchers. Modern prospective dentin / remineralization studies require measurement of the smallest alternations in the mineral content of tooth. One of the techniques used is the energy dispersive x-ray analysis (EDX) attached to scanning electron microscope (SEM). This is an easy, quick and accurate in vitro microanalytical method used for ultrastructural elemental analysis to quantitatively determine the mineral content in a specific tooth sample [6].

Newly developed remineralizing agents were introduced as sodium calcium phosphosilicate (bioactive glass) ceramic material. A toothpaste named NovaMin was introduced based on this formulation.

Since the level of the minerals concentration in enamel is a good indication of the demineralization and/or remineralization [7]. Apart of remineralization, it was interesting to detect the amount of minerals loss after bleaching and their possible gain after application of remineralizing agents. Thus, the aim of the current study was to test over the counter bleaching agents used according to the manufacturer's instructions and/or abused by being frequently repeated and after remineralizing agent application by the aid of the EDX (Energy Dispersive X- Ray spectroscopy).

MATERIALS & METHODS:

Ethical issue: Ethical approval was obtained following the guidelines of the institutional Review Boards (IRB) by the Ethics Committee of the Faculty of Dentistry, Tanta University, Egypt. All patients signed an informed consent sheet upon participation of their extracted incisor teeth in this study.

Trial design: A randomized, controlled, statistication with 3 parallel groups. Randomization with a 1:1:1 allocation ratio was performed. Throughout this trial, Consolidated Standards of Reporting Trials (CONSORT) guidelines were performed.

Sample collection: 30 sound human premolar teeth free from any enamel defects which extracted for orthodontic treatment and were collected from patients aged (30-50) and stored in natural saliva until the time of the test.

Sample size and calculation: Sample size was estimated assuming 5% alpha and 80% study power. The minimum sample size was calculated for detecting a moderate effect size. The total sample size required = number of groups x number per group = 10 x 3 = 30.

The sample size for this study was calculated according to Arkin, 1984 using the following equation:

$$N = \frac{(Za)^2 \times (SD)^2}{(d)^2}$$

N = Total sample size

$Z\alpha$ = Is standard normal variate and its equal 5.23 at $P < 0.05$

SD = Standard deviation of variable

d = Absolute error or precision

Za	*SD*	*d*
5.67	2.26	2

The criteria used for sample size calculation were as follows:

95% confidence limit

86% power of the study

Intervention: The coronal portion of each tooth was separated by using a cylindrical disc with cooling system. The center of the middle third of the labial surface of all samples was covered with adhesive tape with dimensions 3mm × 3mm. All the enamel of the labial surface was protected with transparent acid resistant nail varnish except the created window of labial enamel with dimensions (3mm × 3mm). The amount of minerals on the labial enamel window of all samples was determined by weight using the EDX. The prepared samples were randomly divided into three equal groups (n= 10), **Group I:** the Deluxe home whitening kit was applied on the labial enamel window 1 hour once daily for fourteen days (fourteen cycles) according to the manufacturer instructions. After each bleaching cycle, each sample was rinsed by distilled water then stored in artificial saliva till the next bleaching cycle. The amount & type of minerals were measured by weight using EDX. The samples were subjected to the remineralizing agent NovaMin® (Bioactive glass) on the same labial enamel window for 10 minutes according to manufacturer instructions. **Group II:** The same procedures which have been previously done in group I was followed except for the bleaching regimen which was done according to a proposed patients abuse of 2 hours daily for seven successive days (14 cycles). **Group III:** The samples were subjected to bleaching by applying the same bleaching agent for an hour twice daily for 3 days and 1 hour in the fourth day, which was a total of seven cycles. By the end of cycles, minerals content was measured. Then the remineraliziling agent was applied and all of these procedures were repeated again for seven more cycles.

All data concerning the type and amount of minerals after bleaching and after remineralization was collected, tabulated and statistically analyzed for the three tested groups by using Mann Whitney test to detect any difference using (SPSS version 20).

RESULTS

All variables were checked for normality using descriptive statistics, graphs, and normality tests. For quantitative data, mean, standard deviation (SD), median, and interquartile range were computed.

The statistical analysis of collected data for fluoride content was illustrated in table (1), phosphate content in table (2) and calcium content in table (3).

Regarding Table (1) F- Test was used to compare the mineral content in the three tested groups at a level of significance $P < 0.05$. Comparing the fluoride content of each group at all testing stages a statistical significant difference was recorded showing that these groups were significantly affected by the bleaching regimen. p value of 0.001 was recorded in the 3 tested groups when base line data vs data collected after bleaching cycles (P1) was compared. With P values of 0.001, 0.001 & 0.004 for groups I, II &III respectively.

Comparing Baseline data vs 14 cycles (P1) it was found that a highly significant difference was recorded for the three tested groups with P values of 0.001.

Concerning P2 (comparison between base line versus remineralization) gp I & II were affected significantly by the remineralizing agent recording P values of 0.043, 0.00 respectively however gp III did not gain any benefit from remineralizing agent recorded a statistical non-significant difference with P value of 0.160. In addition, comparing the bleaching cycling versus remineralization for 10 minutes (P3) all groups recorded a statistical significant difference with P values = 0.011, 0.059 & 0.033 for groups I, II & III respectively.

Concerning the phosphate content, it was illustrated in Table 2 that no statistical significant difference between the three tested groups at base line (P=0.456), however they were affected significantly after bleaching cycles and remineralization recording (P= 0.001).

However, with each group a statistical significant difference was recorded showing that these groups were significantly affected by the bleaching regimen with p values of 0.001, 0.001 &0.003 for groups I, II & III respectively.

Concerning P2 (comparison between base line versus remineralization) gp I & II were affected significantly by the remineralizing agent recording P values of 0.010, 0.001 respectively however gp III did not gain any benefit from remineralizing agent recording a statistical non-significant difference with P value of 0.061. In addition comparing the bleaching cycling versus remineralization for 10 minutes (P3) groups I&II recorded a statistical significant difference with P values = 0.002, 0.036 respectively, while group III recorded non–significant difference with P value= 0.074.

Tukey's test was performed to compare the tested groups together at each stage trying to find out which group was considered responsible for the statistically significant difference detected as shown in Table (3). It was found that group I vs III showed no significant difference at any stage. While I vs II and II vs III did not show any significant difference only at base line with P values of 0.989 & 0.568 respectively. This pointed out that group II was responsible for any significant difference present after 14 cycles or remineralization which indicated that the time of application of bleaching agent (2 hrs daily for successive 7 days) has an obvious effect on the phosphate content level.

Regarding the calcium content following the data illustrated in table (4). It was found that P1, P2 and P3 recorded a significant difference in all tested

TABLE (1) Statistical analysis of the fluoride content after bleaching and remineralizing procedures:

Fluoride	Baseline	14 cycle 1h.	Rem 10 m.	f. test	P. value	P1	P2	P3
G I	1.78 ± 0.44	0.94 ± 0.33	1.41 ± 0.39	11.901	0.001*	0.001*	0.043*	0.011*
G II	2.05 ± 0.35	0.80 ± 0.37	1.14 ± 0.43	28.507	0.001*	0.001*	0.00*	0.059*
G III	1.70 ± 0.52	0.95 ± 0.40	1.40 ± 0.43	6.940	0.004*	0.001*	0.160	0.033*
f. test	1.675	0.480	1.385					
P value	0.206	0.624	0.267					

P1: Baseline vs 14 cycle 1h. ***P2: Baseline vs Rem 10 m.*** ***P3: 14 cycle 1h. vs Rem 10 m.***

TABLE (2) Statistical analysis of the Phosphate content after bleaching and remineralizing procedures:

Phosphate	Baseline	14 cycles 1h.	Rem 10 m.	f. test	P. value	P1	P2	P3
G I	34.65 ± 5.11	22.05 ± 4.51	28.95 ± 4.21	18.617	0.001*	0.001*	0.010*	0.002*
G II	34.32 ± 5.93	15.03 ± 4.96	20.39 ± 5.36	33.585	0.001*	0.001*	0.001*	0.036*
G III	31.89 ± 4.82	23.90 ± 4.41	27.79 ± 4.81	7.273	0.003*	0.001*	0.061	0.074
f. test	0.808	10.199	9.301					
P value	0.456	0.001*	0.001*					

TABLE (3) Tukey test comparing the three tested groups together at base line, after bleaching and remineralization procedures:

Tukey test			
	Baseline	**14 cycle 1h.**	**Rem 10 m.**
I vs II	0.989	0.006*	0.001*
I vs III	0.484	0.649	0.854
II vs III	0.568	0.001*	0.005*

groups. On the other hand, it was found that within each gp a highly statistically significant difference was recorded comparing all tested stages, with P values of 0.001. In addition, after comparison between the three tested groups, there was no statistical-significant difference recorded at base line. This was logic since all groups were collected at the same age group. However, comparing the tested groups after bleaching or remineralization a significant difference was recorded with P values equal 0.001. Thus, it was found that the calcium content of the three tested groups was affected by the bleaching regimen and remineralizing procedures significantly.

Performing Tukey's test comparing gp I vs II, gp I vs III and II vs III as shown in table (5) no significant difference was found related to base line data. However, gp II showed a significant difference vs I or III with P value 0.001 & 0.001 indicating that gp II was responsible for any significant difference present. Thus, the time of application of bleaching agent was the most effective factor in loosing Ca ions from the tooth structure.

DISCUSSION

Bleaching agents affect the lightening of

TABLE (4) Statistical analysis of the calcium content after bleaching and remineralizing procedures:

Calcium	**Baseline**	**14 cycle 1h.**	**Rem 10 m.**	**f. test**	**P. value**	**P1**	**P2**	**P3**
G I	60.37 ± 3.42	41.51 ± 2.69	52.31 ± 2.20	113.301	0.001*	0.001*	0.001*	0.001*
G II	59.42 ± 3.75	29.60 ± 6.51	40.24 ± 6.02	73.819	0.001*	0.001*	0.001*	0.001*
G III	60.11 ± 3.57	45.88 ± 3.43	52.17 ± 3.43	42.061	0.001*	0.001*	0.001*	0.001*
f. test	0.189	34.695	27.222					
P value	0.829	0.001*	0.001*					

TABLE (5) Tukey test comparing the three tested groups together at base line, after bleaching and remineralization procedures:

Tukey test			
	Baseline	**14 cycles 1h.**	**Rem 10 m.**
I vs II	0.825	0.001*	0.001*
I vs III	0.986	0.097	0.997
II vs III	0.901	0.001*	0.001*

discolored tooth structure through decomposition of peroxides into free radicals. The free radicals break down large pigmented molecules in enamel into smaller, less pigmented molecules through either oxidation or reduction reactions [8]. In the current study, the Deluxe home whitening kit was used. It is considered a light agent with low concentration of carbamide peroxide.

Carbamide peroxide may promote changes in the mechanical properties of the enamel due to the action of its by-products, such as urea and oxygen. Free radicals of hydrogen peroxide, a component in which carbamide peroxide degrades, have no specificity and react with and degenerate the dental tissues [9, 10].

One of the possible adverse effects of bleaching products is that the enamel structure may be weakened by oxidation of the organic or inorganic elements [11]. The demineralization process of enamel begins when the pH falls below 5.2 to 5.8. [12-14]

In the current study the human premolar teeth were selected as they are commonly extracted for orthodontic reasons even if they were sound, this allowed the collection of large number of sound teeth to perform the experimental tests, all of a comparable age group.

The selected teeth were stored in artificial saliva trying to mimic the clinical situation. The saliva is considered as a natural source of remineralization restoring the minerals of the demineralized bleached enamel as Shannon et al. reported [15] and it also allows re-hardening of the enamel surface [16].

Jiang et al. 2007 [17] suggested the primary cause of the tooth color shift brought on by dental bleaching is mineral loss rather than the breakdown of chromophores. The subsequent mineral absorption during tooth whitening and the treatment's reversal strongly supports this hypothesis [18].

To measure the elemental loss from tooth structure the EDX was used currently. Recently, using EDX facilitates quantitative measurement of the surface minerals alteration accurately without destruction of the sample which aid in reusing them, thus giving dependent data which enables us to apply these results clinically.

Regarding the present results the 3 bleaching regimes tested showed lowering of mineral contents, which was not regained by remineralization. Lee et al. 2006 [19] and Tao Jiang et al. 2008 [20] suggested that the whitening effect is due to the demineralization of the organic matrix of the enamel rather than the subsequent post-bleaching surface roughness. So Ran Kwon's and Wert's 2015 studies emphasize the relationship between the enamel's HAP crystals and the bleached tooth s optical characteristics. They proposed that the size of the crystals affects the hue of the tooth, the carbonization of the crystals affects the tooth's chroma, and that size and carbonization both affect the tooth's value.

This was conflicted by using CP 10%, a decrease in Ca concentration levels, as well as in Ca and P ratio, was observed for bleached enamel. The relationship between Ca and P is an essential indicator of the remineralization process [21, 22]. The decrease in the level of the two elements could lead to an irreversible alteration, preventing the remineralization process from occurring.

Other studies that used CP 10%, following the manufacturer's norms or the standard application time, verified changes in the bleached enamel. Thus, the abusive use of the bleaching gel for a prolonged period promotes a higher degradation of the organic matrix of the enamel, compromising the structure, causing lighter colors [23-25].

Some authors claimed that the initial bleaching opens up highly pigmented carbon-ring complexes and transforms them into chains with lighter colors. In other words, the current yellow-pigmented carbon double-bond molecules are changed into hydroxyl groups that are colorless, when the active oxygen is drawn to electron-rich regions of stain molecules and cleaves double bonds to lighten color and remove the compound [26]. Hydrogen peroxide's oxidizing effect should be restricted to organic chromophores as it penetrates into the enamel and dentin until it reaches a specific saturation point (whitening threshold), at which point only hydrophilic colorless materials are present. Beyond this point, the lightening action rapidly slows down, and the bleaching process (if allowed to continue) starts to break down the carbon-containing components compromising tooth structure [(2).]

The rough surface created after bleaching produces a more diffuse reflection that makes teeth appear brighter, whereas a smooth surface produces a more specular reflection. Additionally, the light scattering of teeth is significantly influenced by the rise in backscattering of short wavelengths reflected as bluish-white [(27, 28)]. According to several articles, bleached teeth's surface topography changes, support the whitening procedure [(29, 30)]

When we confront the variations observed for element levels, especially Ca and P, and the findings found using x-ray diffraction analyzes, we noticed an incongruence, that is, there was a change in the levels of Ca and P elements, after all the chemical elements are not loose but united in molecules. One explanation would be that the observed changes may be indicating and reinforcing the idea of reversible changes, there is a change in the mineral surface, but it is transient and remineralizing. Thus, it is worth mentioning that in the present study, the specimens were left immersed in artificial saliva throughout the experiment, which probably contributed to reverse the demineralization process, that is, remineralization occurred, however, the level of minerals didn't reach Base line data.

On the other hand, some authors concluded that, although tooth bleaching doesn't create macroscopically visible defects, some studies reported some undesirable microscopic alterations which may include: Changes in the chemical composition of teeth, changes in the mineral content of dental structures such as calcium and phosphate, changes in enamel fluoride content [(31-33)]

In addition, currently it was found that gp II carried the responsibility of the significant difference recorded in potassium and calcium content especially, Thus, based on the results of this study, we can assume the excessive use of CP for too long periods changes the surface of the enamel causing irreversible damage of the mineral structure. It was concluded that using of the bleaching agent for an exceeded time even at low concentration has to be limited [(34)].

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