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Original Research Article

The Effects of Artificial saliva and fluoride varnish on Microhardness of Bleached Enamel with 40% Hydrogen Peroxide-An InvitroStudy

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Abstract: Teeth whitening have been accepted as one of the most conservative treatment methods of discolored teeth, however, these materials have also some complications for teeth. The aim of this study was to investigate the effect of artificial saliva and fluoride varnish on microhardness of bleached enamel with 40% hydrogen peroxide. Twenty intact extracted human anterior teeth were selected. Once the initial preparation of the teeth, they were divided into two groups randomly. Primary microhardness of enamel was measured by Vickers test. Then both groups separately were bleached with 40%hydrogen peroxide, and microhardness was measured again. In the next step, first group was stored in artificial saliva(AS) and fluoride varnish was used in second group. Themicrohardness was measured again. Data were analyzed with Paired T- test at a significant level of $P<0.05$. In both groups, enamel microhardnesswas decreased after bleaching, this reductionwas not significant in the first (AS) group, whileit was significant in the second (fluoride) group ($P= 0.3$ in AS group, $P= 0.03$ in fluoride group).Moreover, the artificial saliva ($P= 0.05$) and fluoride ($P= 0.004$), increased the enamel microhardness after bleaching. Based on this study, artificial saliva and fluoride can increase the enamel micro hardness.
Keywords: Hydrogen peroxide-Microhardness- Fluoride.

INTRODUCTION

The smile is one of the most important factors in social relationships [1]. white teeth increase self-confidence and improve their social eminence [2]. Hence, now a days, patients´ demand have increased for esthetic dentistry treatments [1]. There are different ways to achive this goal, such as composite restorations, ceramic crowns or laminates. But, these treatments can cause loss of tooth structure and change the normal contour of the tooth. In modifying of dental discolorations, tooth bleaching treatments are easier, more acceptable,economical and conservative [4 & 3].

There are three basic methods for whitening vital teeth: In office, At home and bleaching with Over the counter products(OTC) . Bleaching can be done with different materials such as hydrogen peroxide, carbamide peroxide, sodium perborate which can be used with different concentrations [5]. These materials diffuse through the organic matrix of tooth structure due to low molecular weight [1, 6]. The attack of hydrogen peroxide in organic matrix leads to destruction of the long organic chains into colorless short chains during the oxidation process[1].

Some studies reported the complications of bleaching agents 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, topographic changes, increase in enamel porosity, opening enamel prisms, effects similar to etched enamel [3, 7, 8], reducing the enamel microhardness [6, 9,10], opening the dentinal tubules and increasing dentin sensitivity [6], as well as, in some studies, changes in the morphology and composition of the enamel were observed, which were also not limited to the surface and can be seen in the subsurface layers of enamel [8]. Based on the hydrodynamic theory,the movement of fluid within the dentinal tubules stimulatesreceptors and createspain.If bleaching agents are along with desensitising materials such as potassium nitrate and fluoride, this problem is reduced [6].

The fact that topical application of fluoride is effective in progressing and retrieving of the minerals and preventing thereductionof them [11,12]. Fluoride compounds may support the recoveringmicrostructure defects resultedfrom bleaching of the teeth with absorption and deposition of salivary compounds such as calcium and phosphate [13]. In a study, hardness of enamel was reduced in all groups through applying carbamide peroxide and but use of fluoride, and calcium increased the microhardness [14].

In a study in 2012, the effects of brushing with toothpaste, on microhardness of bleached enamel with 15% carbamide peroxide were evaluated. The resultsshowed that brushing immediately and 1-2 hours after bleaching have no effect on the enamel microhardness [7]. Also, in one study, The enamel specimens treated with CCP-ACP topical paste showed smoother surfaces than those used fluoride dentifrice[15]. some studies have been performed on the adverse effects of different bleaching materials on the dental tissues as well as on the effects of mineralized agentson the enamel microhardness, however existing studies have shown different results [2,6,7,10,13-17]. Due to the daily increase of the use of bleaching materials and some of their effects on dental tissues, we decided to assess the effects of these materials on the enamel. Hence, the aim of this study is to investigate the effects of artificial saliva and fluoride on the microhardnessof bleached enamelwith 40% hydrogen peroxide.

MATERIALS AND METHODS

Twenty intact extracted human anterior teeth were selected based on inclusion and exclusion criteria. They were extracted due to the orthodontics, periodontal diseases or missing reasons, so that they were without any cracks, caries, filling and buccal, lingual, incisal wears, without discolorations with internal or external origins of congenital imperfections such as fluorosis in visual examination. The teeth were washed thoroughly after being extracted and were kept in distilled water at room temperature until the tests. The specimens were cleaned by scaling instrument and rubber cap and fluoride-free pumice powder. Teeth were mounted in the self-curing acrylic resin(Acropars,Iran), so that the lingual side of the teeth was into the acryl and the labial side was out of it. The crowns of teeth were polished with diamound polishing disks(Brazil-FGM). Each disk was used for five cases.

Specimens were divided into two groups randomly. The microhardness of baselines was measured by Vickers Hardness Testing Machine , in such that, the area of load entering was the central part of the buccal surface of the tooth. The load was 100 gram per 10 seconds. Three Indents of microhardness were measured in each specimen and the average indent was recorded. Both groups were treated with 40% hydrogen peroxide (Opalescence Boost / ultradent / USA) separately according to the manufacturer's instructions. The buccal surface of each tooth, was covered with 2 mm thickness of this material for 15 minutes separately and then was cleaned with cotton and it was repeated twice, each for 15 minutes to 45 minutes. The gel was removed from the teeth by cotton and washed with distilled water. At this stage, enamel microhardness was measured again,in the same conditions were performed .

In the next phase,first group was stored in artificial saliva for 4 hours,and 5%sodium fluoride varnish (Flor Opal Varnish / Ultradent / USA) was used for the secondgroup,accordingto the manufacturer's instructions. Then the microhardness of both groups were measured again. SPSS 20 software was used for data analyzing and Paired-T test was used to compare the mean microhardness of enamel in the first(AS)and second(fluoride)groups, before and after bleaching and after exposed to fluoride and artificial saliva.

RESULTS

This study aimed to evaluate the effect of artificial saliva and fluoride on microhardness of bleached enamel with 40% hydrogen peroxide .

Table 1: Comparison of enamel microhardnessin both groups, before and after bleaching

Micro Hardness / group	before bleaching Mean±SD	After bleaching Mean±SD	P- value
Artificial saliva	400.1±47.3	366. 5 ± 65	0.3
Fluoride varnish	226.5±32.46	189. 52 ± 32.46	0.03

According to the results presented in Table 1, the average primarymicrohardness of enamel (before bleaching) in the first(AS)group was 400.1 ±47.3 and theenamel microhardnessafter bleaching was 366.5 ± 65, comparing the microhardness of two stages was not significant (P= 0.3). As well as,the average primarymicrohardness of enamel in the second(fluoride) group was 226.5 ± 3.4 and theenamel microhardnessafter bleaching was 189.52_+ 32.46 , while comparing ofmicrohardnessof two stages of these groups was significant (P= 0.03). Then, the average microhardness after bleaching and after esposurein remineralized agentswere compared on the basis of P≤ 0.05 (Table 2).

Table 2: Comparison of enamel microhardness in group, after bleaching and expose to remineralizing material

Micro Hardness Group	After bleaching Mean±SD	Remineralised Material Mean±SD	P- value
Artificial saliva	366.5±65	398±53	0.3
Fluoride varnish	189.52+_ 32.46	249.91±39.08	0.03

Based on the results presented in Table 2, the average microhardness of enamel after bleaching in the frst(AS) group was 366.5 ± 65 and it was 398± 53 after immersion in AS .the average microhardness of enamel after bleaching in the second(fluoride) group was 189.52+_32.46 and it was 249.91+_39.08 after exposed to fluoride,which the differences of microhardnesswere significant in two stages of the two groups (P = 0.05).

DISCUSSION

In the present study, the meanmicrohardness of enamel in the AS group was 400.1 ± 47.3 and in the fluoride group was226.5 ± 3.4. Difference in the initial microhardness is resulted from different degrees of enamel mineralization with local variations of enamel tissue and enhance in the porosity near DEJ area. In addition, the microhardness is reduced from the outer surface to the DEJ [18]. As well as, theenamel microhardness is different in other studies, so that consistency in measuring techniques can be referred in addition to pre-mentioned factors. The use of Vickers hardness test or knoophardness ,the amount and duration of load in enamel microhardness measurement, microhardness measurement of surface or subsurface enamel, using of human or bovine teeth specimens, anterior or posterior teeth, can cause discrepancies in these studies [4]. In this study, microhardness of human teeth was measured by Vickers test, under the force of 100 g per 10 seconds. As well as the factors such as environmental factors, the fluoridation of drinking water, dental age and different habits in some countries could also affect the primary microhardness of enamel [19]. The first part of results showed that applying 40% hydrogen peroxide bleaching material in the offices could reduce the enamel microhardness, so that the reduction was not significant in the AS group while it was significant in thefluoride group. (AS group P= 0.3 and fluoride groupP= 0.03).

The results of the firstgroup(AS) of this study wereconsistentwith the study of Shannon *et al.;* (10%CP Carbamide peroxide) [20], Seghi *et al.;* (10% CP) [21], Cadenaro *et al.;* (38% HP) [22], Suleiman *et al.;* (35%HP) [23], Smidt *et al.;* (15- 16%CP) [24] and study of Davari *et al.;* (10CP and 22%) [2], and they were inconsistent with the results of the secondgroup(fluoride). On the other hand, the results of the first group were inconsistent with results of Feagin *et al.;* [25] Soldani *et al.;* [26], Rodrigues *et al.;* [27], Basting *et al.;* [28], Efeoglu *et al.;* [29], Mcguckin *et al.;* [30], Efeoglua *et al.;* [31], Tezel *et al.;* [32] and a part of Borges *et al.;* studies [9], while they were consistent with the results of the second group(fluoride).

It should be noted that in our study also the reduction of enamelmicrohardnesshas occurred in both groups, in other words,the mineral content of enamel has been partly reduced because hydrogen peroxide had oxidation effect on the enamel organic matrix [5,18]. So thatPotocnik *et al.;* demonstrated that the application of 10% carbamide peroxide causes micro structural changes but does not cause changes in enamel hardness [19], this may have also occurred in thiswork in the first group. However, due to the presence of 1.1 % fluoride and 3% potassium nitrate (desensitising agents) in combination with this bleaching material and PH of 7, the effects of bleach on the reduction of enamel microhardness have been modified.SinceAttin *et al.;* [13] showed that the addition of fluoride to the bleaching agentsleads to re-hardening of the bleached enamel.

The reasons for conflicting with other studies include the following: different bleaching materials, differences in PH level [24], the frequency and duration use of bleaching material, oxidative effects of bleaching agents, the amount of bleaching thickeners for stability of the material [33], the presence of fluoride and desensitising agents [34], the variation in the morphology of the enamel [22], differing experimental tests and dental specimens (human / bovine) [4] and the less number specimens [2]. The results showed that the use of fluoride at the second group and use ofartificial saliva in the first group after bleaching of teeth, lead to an increase in microhardness,whichis significant (AS group P= 0.05 and the fluoride group P= 0.004).

These results werealigned with the results of,Bizhang *et al.;* [4], BurlamaquiPinheiro *et al.;* [6], Borges *et al.;* [9], Borges *et al.;* [10], Attin *et al.;* [13],Chen *et al.;* [16]. The presence of fluoride ions around teeth leads to deposit the fluoro- apatite from calcium and phosphate ions in saliva, and fluorideincreases the resistance of teethtissue against demineralization. As well as fluoride wasreplaced by dissolved ions containing magnesium and carbonate which were lost during the demineralization, and this displacementprocess causes more resistant of the enamel.The presence of high concentrations of fluoride level may act as a reservoir for fluoride that promotes remineralization [35]. However, the results of this workwas inconsistentwiththe study ofDavari *et al.;* [2]. So that in the study ofDavarithe use of fluoride toothpaste at a concentration of 1450ppm did not increase themicrohardness after bleaching with 10 % carbamide peroxide.While in the same work ,using the same toothpasteincreased the enamel microhardnessafter bleaching with 22%carbamide peroxide [2]. In this regard, according to Ulukapi *et al.;* [36] and Davari *et al.;* [2] it can be noted that higher concentrations ofcarbamide peroxidedue to more solubility properties of minerals in the enamel, increase the potential of fluoride absorbtion.

In addition, our survey also was in conflict with another part of the Davari et alstudy.So that in the study ofDavarithe application of Tooth Mousse paste containing PPM 900 fluoride decreased the enamel microhardness after bleaching with22% carbamaide peroxide and it wasineffectivein the use of 10% carbamaideperoxide,that's probably due to the presence of 0.2- 0.3 % phosphoric acid inTooth Mousse paste. The storage of the specimens in the artificial saliva led to enhance microhardness. Formulation of artificial saliva used in this study was according to the study of Amaechi *et al.;* [37] with PH of 7. This study was consistent with Amaechi *et al.;* [36], and Gelhard *et al.;* [38], Lussi *et al.;* [39]studies. Artificial saliva contains chemical compounds such as potassium chloride, magnesium chloride, calcium chloride, di potassium hydrogen phosphate, potassium dihydrogen phosphate, which may improve the enamel remineralization [18]. While it was inconsistent with some other studies, including Borges *et al.;* [10] (in their studies the artificial saliva had no effects on enamel hardness), which the conflict was associated with the difference in artificial saliva formula, immersion time, the type of teeth and different study designs. It should be noted that with P = 0.05 in this study, if more specimens were tested, the results would be also altered.

CONCLUSIONS

Although the present study couldn't completely mimic oral environment, the results showed that bleaching agents used in this research can reduce the microhardness of enamel.Also, the Fluoride and artificial saliva had remineralization effects on the bleached enamel. However, further studies using demineralized agents, associated with the effect of oral environment are required.

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