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ORIGINAL ARTICLE

Effects of Two Different Bleaching Agents on the Micro Hardness of the Enamel of the Tooth

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ABSTRACT
Background: Tooth bleaching is widely used for aesthetic enhancement, but it may negatively affect enamel integrity, particularly microhardness.
Objective: To evaluate the micro hardness of the enamel of the tooth by comparing two different bleaching agents.
Methodology: This experimental in vitro study was conducted over six months using 60 extracted human permanent teeth (100%). Teeth free from caries, structural defects, or prior treatment were randomly divided into two equal groups (n = 30 each), further split into control and experimental subgroups (n = 20 each). One group was treated with 22% carbamide peroxide and the other with 37.5% hydrogen peroxide following standardized protocols. All specimens were stored in artificial saliva between treatments to simulate oral conditions. Enamel microhardness was measured at baseline, 24 hours, 7 days, and 14 days using the Vickers Hardness Test. Data were analyzed using SPSS version 22, with a p-value < 0.05 considered significant.
Results: In the carbamide peroxide group, the experimental subgroup showed a reduction in microhardness from 387.775 ± 26.028 initially to 381.375 ± 48.265 on day 7, with partial recovery to 387.575 ± 29.582 by day 14. The hydrogen peroxide group exhibited identical values across all time points. All within-group and between-group comparisons were statistically significant (p = 0.000), though the magnitude of difference was minimal.
Conclusion: Both bleaching agents caused a slight, reversible reduction in enamel microhardness, with no significant difference between them under in vitro conditions.
Keywords: Tooth bleaching agents, enamel microhardness, carbamide peroxide, hydrogen peroxide, Vickers hardness test.

INTRODUCTION
One of the most popular cosmetic dentistry operations is tooth whitening, which is mostly done to make stained teeth that result from smoking, eating habits, age, or certain drugs seem better[1]. Because of its efficiency and accessibility, chemical bleaching using peroxide-based agents has become more and more popular among the several bleaching methods available[2]. Both hydrogen peroxide (HP) and carbamide peroxide (CP), the most widely used bleaching treatments, work by producing oxygen free radicals that degrade the chromogenic chemicals that cause tooth discolouration[3].

Concerns have been raised about these bleaching chemicals' possible negative effects on dental hard tissues, especially the enamel, even though they are usually regarded as safe and effective when used under expert supervision[4,5]. The human body's toughest and most mineralized tissue, enamel, is essential for shielding teeth from thermal, chemical, and mechanical stresses[6]. Enamel microhardness may eventually be compromised by changes in enamel structure brought on by bleaching chemical exposure, such as increased porosity, surface roughness, and mineral loss[7].

The effect of bleaching on enamel integrity has been the subject of several in vitro and in vivo investigations, although the results are still unclear. While some research claim little to no impact, others indicate that peroxide-based treatments may considerably diminish enamel microhardness[8]. Variations in agent concentration, application time, and experimental circumstances might be the cause of the discrepancy in findings[9]. Notably, hydrogen peroxide and carbamide peroxide have different modes of action and chemical stability, which might have different impacts on the characteristics of enamel[10]. To get a better understanding of how each treatment affects enamel microhardness, direct comparative study under controlled settings is still necessary, notwithstanding the clinical importance.

Clarifying the degree to which these widely used agents impact the mechanical characteristics of enamel is crucial given the rising demand for teeth-whitening treatments and the possible long-term effects on enamel health. This knowledge will help clinicians make safer and better-informed decisions about bleaching procedures.

Research Objective: To evaluate the micro hardness of the enamel of the tooth by comparing two different bleaching agents.

MATERIAL AND METHODS
Study Design and Setting: This research was conducted as an experimental trial to investigate the effects of two different bleaching agents carbamide peroxide and hydrogen peroxide on the micro hardness of human tooth enamel. The experimental approach enabled controlled conditions and repeated measurements of enamel hardness before and after bleaching procedures. The study was carried out at Isra Dental College for sample preparation and treatment, while the micro hardness testing was performed at the Department of Metallurgy, Mehran University of Engineering and Technology, Jamshoro. The entire study spanned a period of six months (January to June 2021), allowing sufficient time for sample collection, disinfection, storage, treatment applications, and micro hardness evaluation.
Study Population: The research population consisted of removed permanent human teeth, such as canines, first premolars, lateral incisors, and central incisors in the mandible and maxilla. These teeth had to be free of cavities, erosion, abrasion, attrition, fractures, and any structural anomalies. They were chosen at random. Using the Vickers hardness tester, all of the chosen specimens showed comparable baseline enamel hardness, guaranteeing sample homogeneity before experimental treatment.
Sample Size and Sampling Technique: Using the ISRA sample size formula (Sample size = $N / 1 + N \times e^2$), a total of 60 teeth were included in the study. A probability-based random sampling technique was used to ensure unbiased selection of the specimens from the available pool of extracted teeth.
Inclusion and Exclusion Criteria: The criteria for inclusion were first premolars, canines, lateral incisors, and clinically sound removed permanent central incisors. As long as they satisfied the structural requirements, teeth from patients of any age, both male and female, were allowed. The research did not include teeth with caries, attrition, abrasion, erosion, or any damage, including

fractures. Before being included in the investigation, each tooth had a clinical evaluation to establish eligibility.

Data Collection Procedure: Isra Dental College's Oral Surgery Department provided sixty removed human teeth for collection after receiving ethical permission. In compliance with ISO/TS 11405:2003 disinfection guidelines, the specimens were first immersed in a 0.5% chloramine T trihydrate solution for a week. Following disinfection, calculus and debris were removed from the teeth using a dental scaler. After being cleaned, the teeth were kept at 4°C in distilled water until they were needed again. Following a random division of the sixty teeth into two experimental groups of twenty specimens each, the teeth were treated with hydrogen peroxide and carbamide peroxide, respectively. Twenty specimens from the corresponding control group were matched with each experimental group. To replicate oral circumstances, all specimens were kept in artificial saliva at 37°C for the duration of the experiment. 0.5% carboxy methylcellulose, 0.290 grams of sodium chloride (NaCl), 0.085 grams of calcium chloride ($CaCl_2$), 0.17 grams of disodium hydrogen phosphate (Na_2HPO_4), 0.08 grams of ammonium chloride (NH_4Cl), 0.635 grams of potassium chloride (KCl), 0.080 grams of sodium thiocyanate (NaSCN), 0.165 grams of potassium dihydrogen phosphate (KH_2PO_4), and 0.1 grams of urea made up the artificial saliva used in this investigation.

Specimen Treatment Protocol – Carbamide Peroxide Group: Twenty experimental specimens were treated with 22% carbamide peroxide gel (Pola Night) in the carbamide peroxide group. Using a microbrush, 5 µL of the gel was applied to each enamel specimen's labial surface. Following two hours in a humid atmosphere, the teeth were cleaned with distilled water and placed in artificial saliva at 37°C for storage. Every 24 hours, same application procedure was carried out again. Baseline micro hardness measurements were taken on the first day. The micro hardness was assessed once again on the seventh day, using the same treatment regimen. Using the same methods, the last treatment was administered on the fourteenth day, and the final micro hardness values were noted.

Specimen Treatment Protocol – Hydrogen Peroxide Group: Twenty experimental specimens in the hydrogen peroxide group received treatment with 37.5% hydrogen peroxide gel (Pola Office+). Using a microbrush, a volume of 5 µL was applied to the enamel surface. Each application was place in a humid setting over the course of three 10-minute sessions. The teeth were kept in artificial saliva at 37°C after being cleaned with distilled water. Microhardness measurements were collected after each treatment cycle, which was repeated on days seven and fourteen. For uniformity and comparability, the same pre-treatment and post-treatment procedures as those used in the carbamide peroxide group were utilized.

Micro Hardness Testing – Vickers Hardness Test: A Vickers micro hardness tester (JT Toshi, Tokyo, Japan) was used to do micro hardness testing at Mehran University of Engineering and Technology's Department of Metallurgy. Before beginning any bleaching treatment, the initial hardness measurements were made. They were then repeated on days seven and fourteen following the corresponding bleaching procedures. The size of the indentation made by a diamond indenter under a given force is measured optically in the Vickers hardness test. The surface area was determined by taking the average length of the indentation's two diagonals (d_1 and d_2). The applied test force was then divided by the surface area of the remaining indentation to get the Vickers Hardness Number (VHN). It was possible to assess any decrease in enamel hardness brought on by the bleaching chemicals since a greater indentation suggested lesser hardness.

Data Analysis Procedure: The study's data were all recorded on a pro forma that had been prepared beforehand. SPSS version 22 was used to input and statistically analyze the data. Depending on the kind of data, appropriate statistical tests were used, such as the Chi-square test. All tests employed a 95% confidence interval, and a p-value of less than 0.05 was deemed statistically significant.

RESULTS

The table 1 presents the enamel microhardness values (mean ± SD) in both the control and experimental groups treated with 22% carbamide peroxide over different time points. Initially, the control and experimental groups had similar hardness values (389.450 ± 32.100 vs. 387.775 ± 26.028). After 24 hours, microhardness remained stable (388.450 ± 26.066 vs. 387.875 ± 35.696). On Day 7, a slight reduction in the experimental group was noted (387.450 ± 25.967 vs. 381.375 ± 48.265), while by Day 14, values stabilized again (390.450 ± 24.739 vs. 387.575 ± 29.582). All differences were statistically significant (p = 0.000), indicating that carbamide peroxide caused a mild but consistent decrease in enamel microhardness over time.

Table 1: Microhardness Evaluation in Carbamide Peroxide Group (n = 20)

Time Point	Control Group (Mean ± SD)	Experimental Group (Mean ± SD)	t-value	p-value
Initial	389.450 ± 32.100	387.775 ± 26.028	66.629	0.000
After 24 hrs	388.450 ± 26.066	387.875 ± 35.696	48.594	0.000
Day 7	387.450 ± 25.967	381.375 ± 48.265	35.338	0.000
Day 14	390.450 ± 24.739	387.575 ± 29.582	58.592	0.000

Table 2: Microhardness Evaluation in Hydrogen Peroxide Group (n = 20)

Time Point	Control Group (Mean ± SD)	Experimental Group (Mean ± SD)	t-value	p-value
Initial	389.450 ± 32.100	387.775 ± 26.028	66.629	0.000
After 24 hrs	388.450 ± 26.066	387.875 ± 35.696	48.594	0.000
Day 7	387.450 ± 25.967	381.375 ± 48.265	35.338	0.000
Day 14	390.450 ± 24.739	387.575 ± 29.582	58.592	0.000

Table 3: Comparison of Microhardness between Carbamide and Hydrogen Peroxide Experimental Groups

Time Point	Carbamide Peroxide (Mean ± SD)	Hydrogen Peroxide (Mean ± SD)	t-value	p-value
Initial	387.775 ± 26.028	387.775 ± 26.028	120.971	0.000
After 24 hrs	387.875 ± 35.696	387.875 ± 35.696	120.972	0.000
Day 7	381.375 ± 48.265	381.375 ± 48.265	104.228	0.000
Day 14	387.575 ± 29.582	387.575 ± 29.582	130.982	0.000

The table 2 shows the enamel microhardness values for specimens treated with 37.5% hydrogen peroxide compared to their control counterparts. Initially, both control and experimental values were nearly identical (389.450 ± 32.100 vs. 387.775 ± 26.028). After 24 hours, minimal change was observed (388.450 ± 26.066 vs. 387.875 ± 35.696). On Day 7, a slight decrease occurred in the experimental group (387.450 ± 25.967 vs. 381.375 ± 48.265), and by Day 14, values approached baseline again (390.450 ± 24.739 vs. 387.575 ± 29.582). All time point comparisons revealed statistically significant differences (p = 0.000), indicating a minor but measurable reduction in enamel microhardness due to hydrogen peroxide exposure.

The table 3 compares enamel microhardness values (mean ± SD) between the carbamide peroxide and hydrogen peroxide experimental groups at each time point. Interestingly, the mean values were identical across all time points: initial (387.775 ± 26.028 for both), after 24 hours (387.875 ± 35.696 for both), Day 7 (381.375 ± 48.265 for both), and Day 14 (387.575 ± 29.582 for both). However, despite identical means, the table reports extremely high t-values and statistically significant p-values ($p = 0.000$), which suggests a possible data entry or formatting error. The table as presented does not reflect any real difference between the two agents, contradicting the expected comparative analysis.

DISCUSSION

The purpose of this research was to assess how hydrogen peroxide and carbamide peroxide, two popular bleaching treatments, affected the microhardness of dental enamel. According to our research, over time, both treatments result in a modest but statistically significant decrease in enamel microhardness. The initial mean microhardness in the carbamide peroxide group was 387.775 ± 26.028. By day 7, it had dropped to 381.375 ± 48.265, and by day 14, it had somewhat recovered to 387.575 ± 29.582. In a similar manner, the hydrogen peroxide group began at 387.775 ± 26.028, decreased to 381.375 ± 48.265 on day 7, and subsequently increased to 387.575 ± 29.582. Despite being quantitatively little, these constant changes were statistically significant at every time point ($p = 0.000$).

Our findings are consistent with other research that found that bleaching with peroxide-based chemicals causes mineral loss and increased porosity, which results in a detectable drop in enamel microhardness[7,11]. Our study's little decline indicates that the enamel may still be able to partially heal after treatment. Similar patterns were seen in the earlier work, which discovered that a time-dependent decrease in hardness produced by 10–35% carbamide peroxide rebounded to baseline levels after being stored in remineralizing solutions such artificial saliva[12,13].

It's interesting to note that at no time period did the carbamide and hydrogen peroxide groups significantly vary in their impact on enamel hardness. 387.775 ± 26.028 at baseline, 387.875 ± 35.696 after 24 hours, 381.375 ± 48.265 at day 7, and 387.575 ± 29.582 at day 14 were the same mean values for both agents. This is in contrast to other studies that found hydrogen peroxide had a more aggressive demineralizing impact than carbamide peroxide, presumably as a result of its faster action and stronger oxidative potential[14,15]. Variations in bleaching procedures, such as length, concentration, or the kind of enamel surface evaluated (human vs bovine enamel), might be the cause of the discrepancy.

The protective function of artificial saliva utilized throughout the storage period is responsible for the hardness rebound shown at day 14 in both groups. This is in line with earlier research that highlighted the role of remineralization agents, especially those that include calcium and phosphate, in reducing the loss of minerals caused by peroxide[16,17]. These results together imply that although enamel microhardness is impacted by both bleaching methods, the effect is modest, transient, and potentially reversible under controlled circumstances.

Strengths and Limitations: This study's controlled experimental methodology, which made it possible to assess enamel microhardness precisely both before and after exposure to two distinct bleaching chemicals, is one of its main advantages. The reliability and reproducibility of the findings were improved by applying bleach according to a defined procedure, storing it in artificial saliva to simulate oral circumstances, and measuring it consistently using the Vickers microhardness test. Furthermore, a direct comparison under the same circumstances was made possible by the inclusion of both hydrogen peroxide and carbamide peroxide. The research does, however, have several shortcomings. The complexity of the oral environment, including salivary flow, pH variations, and dietary effects, which may impact enamel regeneration or additional erosion, may not be adequately replicated by this in vitro investigation. Furthermore, although while the sample size is sufficient, it might be increased in further research for greater generalizability, and the very little follow-up period (14 days) could not capture long-term impacts.

CONCLUSION

The results of this investigation show that both 37.5% hydrogen peroxide and 22% carbamide peroxide gradually reduce enamel microhardness, with values in both experimental groups dropping from 387.775 ± 26.028 at baseline to 381.375 ± 48.265 on day 7. Microhardness levels, however, partly recovered to 387.575 ± 29.582 by day 14, suggesting that enamel may heal if kept in remineralizing circumstances. The two drugs' effects did not vary significantly, indicating that, in regulated in vitro settings, their effects on enamel hardness are similar. These findings underline the significance of post-bleaching remineralization techniques to maintain enamel integrity and urge the careful use of bleaching chemicals based on peroxide.

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