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# The effect of ozone and silver diamine fluoride combination on enamel microhardness: an *in vitro* study

Efeito da combinação de ozônio e diamino fluoreto de prata na microdureza do esmalte: um estudo *in vitro*

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## ABSTRACT

**Objective:** This study aims to evaluate ozone's impact on initially formed enamel caries before applying silver diamine fluoride, utilizing a Vickers microhardness tester as the excitation source. **Material and Methods:** Sixty premolars were exposed to demineralization to form artificial incipient caries. Then, the teeth were arbitrarily distributed among four investigational groups (group 1 received silver diamine fluoride, group 2 received ozone, group 3 received ozone then silver diamine fluoride, while group 4 received no treatment as a control group). Enamel microhardness was evaluated at three stages: before exposure to demineralization solution, after exposure, and following treatment using a Vickers microhardness tester. A one-way ANOVA test was utilized to calculate the difference in microhardness values across the four groups, followed by Tukey's test and paired t-tests were accomplished before and after treatment. 5% was established as the significance level. **Results:** The data shows a clear and statistically significant difference in surface microhardness between the control and experimental groups. Tukey's test indicates that Group 3 and Group 1 did not have a significant difference. However, paired t-tests conducted before and after treatment revealed significant differences in the experimental groups, while non-significant differences were found before and after in the control group. **Conclusion:** This investigation disclosed that SDF is still an excellent option whereas ozone alone has no impact on remineralizing enamel incipient caries, and there is no advancement in combining both approaches.

## KEYWORDS

Dental caries; Fluorides; Hardness; Ozone; Silver diamine fluoride.

## RESUMO

**Objetivo:** Esse estudo tem como objetivo avaliar o impacto do ozônio na formação de cárie inicial em esmalte previamente a aplicação de diamino fluoreto de prata, foi utilizado microdurômetro Vickers como fonte de análise. **Material e Métodos:** Sessenta pré-molares foram expostos a desmineralização para formação artificial de lesões incipientes de cárie. Então, os dentes foram arbitrariamente distribuídos entre quatro grupos de investigação (grupo 1 – diamino fluoreto de prata; grupo 2 – ozônio; grupo 3 – ozônio seguido de diamino fluoreto de prata; grupo 4 – sem tratamento, grupo controle). A microdureza do esmalte foi avaliada em três estágios: antes da desmineralização, após a desmineralização e após a aplicação dos tratamentos, utilizando um microdurômetro Vickers. Teste ANOVA a um fator foi utilizado para avaliar a diferença nos valores de microdureza entre os quatro grupos, seguido do teste de Tukey e teste T pareado para contemplar antes e após o tratamento. 5% foi estabelecido como nível de significância. **Resultados:** Os dados mostram clara e significante diferença estatística na microdureza superficial entre os grupos controle e experimentais. Teste de Tukey indica que o grupo 3 e grupo 1 não tiveram diferença significativa. No entanto, o teste T pareado conduzido antes e após o tratamento revelou significante diferenças entre os grupos experimentais, enquanto nenhuma diferença significante foi encontrada

antes e após o tratamento para o grupo controle. **Conclusão:** Essa investigação mostra que o diamino fluoreto de prata ainda é uma excelente opção enquanto o ozônio sozinho não tem impacto na remineralização do esmalte em lesões incipiente de cárie, e não apresentou melhora nos resultados com a combinação das técnicas.

## PALAVRAS-CHAVE

Cárie dentária; Fluoretos; Dureza; Ozônio; Diamino fluoreto de prata.

## INTRODUCTION

Fluoride has been the most effective remineralization method in clinical settings for several decades. However, in the last few years, new remineralizing approaches have been created that integrate phosphates and calcium at the level of demineralized tooth surfaces [1].

Tooth decay has been described to be a multifactorial sugar-driven, biofilm-mediated, multifactorial, dynamic disease [2], posing a significant economic challenge to governments' healthcare systems [3]. Management of tooth caries concentrates on applying remineralizing agents to the initial caries for promoting remineralization and controlling demineralization. Remineralizing means creating a super-saturated situation close to the dissolved carious lesion, consequently, it would stop mineral loss and fill up the empty spaces with calcium and phosphate ions [4,5].

Fluoride is known to be a remineralizing agent that forms fluorapatite by incorporating calcium and phosphate ions and reacting with saliva on the interface between the enamel and subsurface areas of teeth [6,7]. One of these agents is silver diamine fluoride (SDF) which is a cariostatic agent [8]. Its use might be widely encouraged as a preventive substitute for the traditional invasive decay treatment approach, particularly for children's patients with special care needs, those too little to receive dental treatment or patients who have trouble accessing and paying for traditional dental treatment [9].

Since the advancement of minimal dental intervention in caries treatment, Ozone has been presented as an alternate stratagem to support the reversal of the initial caries. It is assumed that ozone-induced oxidative damage opens dentinal tubules, subsequently aiding in the remineralization of decay [10]. Low certainty of evidence and poor support from published articles especially systematic reviews concerning the capability of ozone in supporting remineralization with or without the existence of further remineralizing agents demands further research [11,12]. Thus, the current study aimed to weigh the effect of applying ozone on artificially formed initial enamel caries before applying SDF in the microhardness values.

## MATERIAL AND METHODS

### Tooth preparation

The research ethics committee at the College of Dentistry /University of Baghdad approved the protocol of this study (project No.723322) on December 28, 2022. Caries-free extracted premolars were selected for orthodontic purposes with informed consent obtained beforehand. Before testing, the premolars were polished with pumice to remove contaminants or debris on the tooth surfaces and then kept at room temperature in a 0.1% thymol solution. Using a stereomicroscope at ×40 magnification, the teeth were examined, and any tooth with white spot lesions, stains, or cracks was excluded. Finally, sixty premolars were coated with acid-resistant nail paint, leaving a window of approximately 6mm in diameter in the middle of each buccal enamel surface.

### Demineralization and treatment procedure

Artificial lesions were created on the teeth by using a demineralizing solution containing 2.2 mM $NaH_2PO_4$, 2.2 mM $CaCl_2$, and 0.05 M acetic acid for 96 hours [13]. During this process, the pH of the solution was adjusted to 4.4 with 1M KOH and refreshed daily to maintain the pH level.

After air-drying the demineralized surfaces, the teeth were arbitrarily distributed among four investigational groups (n=15):

Group 1, the SDF group, was treated by rubbing SDF solution on the exposed enamel surface for two minutes using a micro brush. The SDF solution used was 38% SDF Advantage Arrest™ from Elevate Oral Care LLC., West Palm Beach. After the treatment, the teeth were flushed with distilled water for thirty seconds [14]. A few days later after SDF application, grayish staining was seen. The staining was caused by SDF because of the presence of the silver ion.

Group 2, the Ozone group, was treated by applying ozone for 80 seconds and then rinsing it off for 15 seconds [15]. The ozonated water used in the treatment was obtained from an Ozone Generator called Aqua-8.

Group 3, the Ozone and SDF group, received ozone treatment followed by SDF application.

Group 4, Control group, received no treatment and was only flushed with deionized water.

### Surface microhardness measurement

Premolars were assessed for microhardness of the enamel surface at three different stages. Firstly, immediately after they were removed from the solution (distal water+2% thymol) at room temperature (baseline). Secondly, after being removed from the demineralization solution. Lastly, after being treated with the remineralizing agents. Three different indentations were made with a Vickers microhardness tester, and the results were averaged under a 500 g load for 30 seconds. The hardness variables were calculated using the following formula:

$$\Delta VHN = VHN\ remineralization - VHN\ demineralization \quad (1)$$

### Statistical analysis

SPSS Statistics version 26.0 software (IBM Corporation, Armonk, NY, USA) was utilized to examine the data. To determine if there was a difference in ΔVHN among the four groups (SDF, ozone, ozone and SDF, and control groups), one-way ANOVA tests were performed, afterwards Tukey's test was used. Also, paired t-tests were accomplished before and after treatment, 5% has been selected as the significance level.

## RESULTS

A remarkable decrease in the surface microhardness was noticed after demineralization then it increased again after treatment. A non-significant difference was obtained in the microhardness unit (VHN) between groups (p= 0.103) at the baseline. Nevertheless, after treatment, a significant difference was found between them (p=0.000).

Concerning ΔVHN, group 3 exhibited the highest mean (91.00 ± 8.13) and group 1 was close to it (86.84 ± 3.038), while the lowest mean was for group 4 (0.93 ±5.15). A significant difference was obtained in the surface microhardness among the experimental and the control groups (p=0.000). Using Tukey's test, significant differences were found between all groups except between group 1 and group 3 where there was no significant difference (P=0.296).

Using paired t-tests for each group before and after treatment, significant differences were obtained with the groups except for the control group, no significant difference was found (P=0.494), as illustrated in Table I.

## DISCUSSION

This study evaluated the effectiveness of SDF, ozone, and the combined use of ozone and SDF in treating artificial enamel caries. Two methods were used to assess enamel remineralization: the Knoop method and Vickers' hardness [16,17]. Among the two, the latter was deemed more

**Table I -** Analysis of VHN among the study groups at different stages by mean ±SD, t-test, and Tukey's test

| Group | Baseline | Demineralization | Remineralization | T-test | ΔVHN |
|---|---|---|---|---|---|
| | Mean ±SD | Mean ±SD | Mean ±SD | p value | Mean ±SD |
| 1 | 301.26 ± 6.71 | 152.49± 2.77 | 239.33 ± 3.66 | 0.000 | 86.84 ± 3.038* |
| 2 | 300.96 ± 6.51 | 144.67 ± 8.16 | 177.30 ± 7.99 | 0.000 | 32.62 ± 7.92 |
| 3 | 303.51 ± 2.03 | 151.36 ± 7.63 | 242.37 ± 1.63 | 0.000 | 91.00 ± 8.13* |
| 4 | 305.33 ± 5.03 | 140.31 ± 6.89 | 141.24 ± 10.97 | 0.494 | 0.93 ±5.15 |

*Tukey's test revealed non-significant difference between group 1 and group 3 only (p>0.05).

suitable because it enables easy measurement and detection of the pyramid-shaped marks on the enamel surface, both visually and digitally [18].

According to research by Godoi et al. [19], the use of SDF treatment led to an increase in mineral density for experimentally created caries, which aligns with the findings of the current investigation. The analysis discovered that the group treated with SDF had a greater effect on remineralizing enamel caries in comparison to the ozone alone and control groups.

However, Akyildiz and Sönmez [20] researched the remineralization capacity of SDF and NaF on artificial caries of the third molar enamel and concluded that while both SDF and NaF can remineralize carious enamel lesions, NaF was more effective. Also, a study done by Alcorn et al. [21] found that fluoride varnish might be a better option than SDF to treat noncavitated, developing, white-spot, carious enamel lesions.

The result disclosed that the adjunctive function of ozone and SDF had a comparable remineralizing effect to SDF alone on carious enamel lesions. These findings were consistent with Yu et al.'s [22] research, which demonstrated that the supplementary use of NaF varnish and SDF had a comparable remineralizing impact on SDF on enamel lesions.

Conversely, Zhao et al. [23] discovered that enamel blocks treated with a laser mixed with SDF had significantly higher hardness than those receiving only SDF or laser. Furthermore, the research of Grocholewicz et al. [24], and Samuel et al. [25] suggested that the use of ozone and nano-hydroxyapatite treatment has the potential for remineralizing carious enamel, but the combination of the two approaches delivers the best outcome.

There was a considerable difference in hardness before and after the application of ozone group, which was only higher than the control group. However, when combined with SDF, a better effect was obtained. This is consistent with another study that found therapy with ozone alone was ineffective in preventing enamel from demineralization or stimulating remineralization unless paired with the Reductant/ Patient Kit, which had a significant amount of fluoride [26]. On the other hand, Atabek and Oztas [27] confirmed that ozone, individually and in combination with other remineralization treatments, helped reverse incipient fissure caries. Similarly, Floare et al. [28] observed that ozone could enhance the enamel condition with substantial shifts in the demineralization values from equivalent early demineralized enamel levels to that approximated to the values of integral enamel, deprived of the risk of a carious lesion procedure. Besides, Srinivasan et al. [29] found that ozone could be regarded as an efficient agent in the reversal of the incipient carious enamel lesion there through preventing the dental tissue from submitting to the recurring restorative cycle.

Nevertheless, this in vitro study had definite restrictions, such as the challenge in replicating the mouth and the fact that carious lesion remineralization is different in comparison to the complicated biological systems in the mouth [30]. In addition, the antimicrobial effect of the two examined agents was not considered, although it could potentially improve the clinical remineralization of decay in the presence of free mineral ions and the antibacterial activeness of the examined agent at the same time.

## CONCLUSION

This investigation disclosed that SDF is still an excellent option whereas ozone alone has no impact on remineralizing enamel incipient caries, and there is no advancement in combining both approaches.

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None.

## Author's Contributions

AMK: Conceptualization, Methodology, Software, Investigation, Writing – Original Draft Preparation, Project Administration and Funding Acquisition. SAA: Methodology, Review & Editing, Project Administration and Funding Acquisition. AHMJAH: Formal Analysis, Review & Editing, Visualization, Supervision, Project Administration and Funding Acquisition. MSMA: Resources, Data Curation, Writing, Project Administration and Funding Acquisition.

## Conflict of Interest

The authors have no conflicts of interest to declare.

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## Regulatory Statement

The research ethics committee at the College of Dentistry /University of Baghdad approved the protocol of this study (project No.723322) December 28, 2022.

## REFERENCES

1. Lazar L, Vlasa A, Beresescu L, Bud A, Lazar AP, Matei L, et al. White Spot Lesions (WSLs)—Post-orthodontic occurrence, management and treatment alternatives: a narrative review. J Cli Med. 2023;12(5):1908. https://doi.org/10.3390/jcm12051908.
2. Pitts NB, Baez RJ, Diaz-Guillory C, Donly KJ, Alberto Feldens C, McGrath C, et al. Early childhood caries: IAPD Bangkok Declaration. J Dent Child (Chic). 2019;86(2):72. PMid:31395110.
3. Turki OH, Jafar ZJ. Antibacterial activity of *Juglans regia* L. Dry Husk Extract against *Streptococcus mutans* and *Lactobacillus*: an in vitro study. Dent Hypotheses. 2023;14(1):29-31. http://doi.org/10.4103/denthyp.denthyp_144_22.
4. Chokshi K, Chokshi A, Konde S, Shetty SR, Chandra KN, Jana S, et al. An in vitro comparative evaluation of three remineralizing agents using confocal microscopy. J Clin Diagn Res. 2016;10(6):ZC39-42. http://doi.org/10.7860/JCDR/2016/18191.7984. PMid:27504408.
5. Hassan NM, Jafar ZJ, Abdul Latif MH. Nano-hydroxyapatite preparation for the remineralization of primary tooth enamel surface subjected to liquid medication: an observational study. Health Sci Rep. 2023;6(4):e1188. http://doi.org/10.1002/hsr2.1188. PMid:37056466.
6. Fischer RB, Muhler JC, Wust CJ. Effects of several fluoride reagents on the surface structure of powdered dental enamel. J Dent Res. 1954;33(1):50-4. http://doi.org/10.1177/00220345540330011201. PMid:13130761.
7. Kanduti D, Sterbenk P, Artnik B. Fluoride: a review of use and effects on health. Mater Sociomed. 2016;28(2):133-7. http://doi.org/10.5455/msm.2016.28.133-137. PMid:27147921.
8. Seifo N, Cassie H, Radford JR, Innes NPT. Silver diamine fluoride for managing carious lesions: an umbrella review. BMC Oral Health. 2019;19(1):145. http://doi.org/10.1186/s12903-019-0830-5. PMid:31299955.
9. Fung MHT, Wong MCM, Lo ECM, Chu CH. Arresting early childhood caries with silver diamine fluoride-a literature review. J Oral Hyg Health. 2013;1(3):1000117. http://doi.org/10.4172/2332-0702.1000117.
10. Ahmmad BZ, Ibraheem LM. Anti-microbial effect of different timeâ€™ sexposureofozonized gas and ozonized water onperiodontalpathogens (in vitro Study). Journal of Baghdad College of Dentistry. 2017;29(2):78-82. http://doi.org/10.12816/0038754.
11. Brazzelli M, McKenzie L, Fielding S, Fraser CM, Clarkson J, Kilonzo MM, et al. Systematic review of the effectiveness and cost-effectiveness of HealOzone for the treatment of occlusal pit/fissure caries and root caries. Health Technol Assess. 2006;10(16):iii-iv, ix-80. http://doi.org/10.3310/hta10160. PMid:16707073.
12. Santos GM, Pacheco RL, Bussadori SK, Santos EM, Riera R, de Oliveira Cruz Latorraca C, et al. Effectiveness and safety of ozone therapy in dental caries treatment: systematic review and meta-analysis. J Evid Based Dent Pract. 2020;20(4):101472. http://doi.org/10.1016/j.jebdp.2020.101472. PMid:33303100.
13. Al-Mamoori RMHJ, Al Haidar AHMJ. Effect of resin infiltration and microabrasion on the microhardness of the artificial white spot lesions (an in vitro study). J Bagh Coll Dent. 2022;34(1):44-50. http://doi.org/10.26477/jbcd.v34i1.3091.
14. Mohammadi N, Farahmand Far MH. Effect of fluoridated varnish and silver diamine fluoride on enamel demineralization resistance in primary dentition. J Indian Soc Pedod Prev Dent. 2018;36(3):257-61. http://doi.org/10.4103/JISPPD.JISPPD_4_18.
15. Hall LH, Cochran MA, Swartz ML. Class 5 composite resin restorations: margin configurations and the distance from the CEJ. Oper Dent. 1993;18(6):246-50. PMid:8152997.
16. Shetty S, Hegde MN, Bopanna TP. Enamel remineralization assessment after treatment with three different remineralizing agents using surface microhardness: an in vitro study. Journal of conservative dentistry. JCD. 2014;17(1):49-52. PMid:24554861.
17. Facio RC, Vieira-Junior W, Basting RT, Turssi CP, do Amaral FLB, França FMG. Influence of fluoride-releasing materials in the inhibition of enamel and dentin demineralization around restorations. Braz Dent Sci. 2022;25(4):e3505. http://doi.org/10.4322/bds.2022.e3505.
18. Heravi F, Ahrari F, Tanbakuchi B. Effectiveness of MI Paste Plus and Remin Pro on remineralization and color improvement of postorthodontic white spot lesions. Dent Res J (Isfahan). 2018;15(2):95-103. http://doi.org/10.4103/1735-3327.226532. PMid:29576772.
19. Godoi FA, Carlos NR, Bridi EC, Amaral FLBD, França FMG, Turssi CP, et al. Remineralizing effect of commercial fluoride varnishes on artificial enamel lesions. Braz Oral Res. 2019;33:e044. http://doi.org/10.1590/1807-3107bor-2019.vol33.0044. PMid:31141037.
20. Akyildiz M, Sönmez IS. Comparison of remineralising potential of nano silver fluoride, silver diamine fluoride and sodium fluoride varnish on artificial caries: an in vitro study. Oral Health Prev Dent. 2019;17(5):469-77. http://doi.org/10.3290/j.ohpd.a42739. PMid:31268047.
21. Alcorn A, Al Dehailan L, Cook N, Tang Q, Lippert F. Longitudinal in vitro effects of silver diamine fluoride on early enamel caries lesions. Oper Dent. 2022;47(3):309-19. http://doi.org/10.2341/20-237-L. PMid:35776957.
22. Yu OY, Mei ML, Zhao IS, Li Q-L, Lo EC-M, Chu C-H. Remineralisation of enamel with silver diamine fluoride and sodium fluoride. Dent Mater. 2018;34(12):e344-52. http://doi.org/10.1016/j.dental.2018.10.007. PMid:30482611.
23. Zhao IS, Xue VW, Yin IX, Niu JY, Lo ECM, Chu CH. Use of a novel 9.3-μm carbon dioxide laser and silver diamine fluoride: prevention of enamel demineralisation and inhibition of cariogenic bacteria. Dent Mater. 2021;37(6):940-8. http://doi.org/10.1016/j.dental.2021.02.017. PMid:33707067.
24. Grocholewicz K, Matkowska-Cichocka G, Makowiecki P, Droździk A, Ey-Chmielewska H, Dziewulska A, et al. Effect of nano-hydroxyapatite and ozone on approximal initial caries: a randomized clinical trial. Sci Rep. 2020;10(1):11192. http://doi.org/10.1038/s41598-020-67885-8. PMid:32636425.
25. Samuel SR, Dorai S, Khatri SG, Patil ST. Effect of ozone to remineralize initial enamel caries: in situ study. Clin Oral Investig. 2016;20(5):1109-13. http://doi.org/10.1007/s00784-016-1710-x. PMid:26759338.
26. Tahmassebi J, Chrysafi N, Duggal MS. The effect of ozone on progression or regression of artificial caries-like enamel lesions in vitro. J Dent. 2014;42(2):167-74. http://doi.org/10.1016/j.jdent.2013.11.011. PMid:24287258.

27. Atabek D, Oztas N. Effectiveness of ozone with or without the additional use of remineralizing solution on non-cavitated fissure carious lesions in permanent molars. Eur J Dent. 2011;5(4):393-9. http://doi.org/10.1055/s-0039-1698911. PMid:22654551.

28. Floare AD, Scurtu AD, Balean OI, Chioran D, Buzatu R, Sava Rosianu R, et al. The biological effects of ozone gas on soft and hard dental tissues and the impact on human gingival fibroblasts and gingival keratinocytes. Processes (Basel). 2021;9(11):1978. http://doi.org/10.3390/pr9111978.

29. Srinivasan SR, Prabhu V, Chandra S, Koshy SS, Acharya S, Mahato KK. Does ozone enhance the remineralizing potential of nanohydroxyapatite on artificially demineralized enamel? A laser induced fluorescence study. Lasers in Dentistry XX. 2014;8929:892903. http://doi.org/10.1117/12.2039399.

30. Torres CRG, Spinola MS. Prado RF, Rodrigues VA, Gutierrez NC, Borges AB. Efficacy of fluoride varnishes with different compositions on white spot lesions remineralization. Braz Dent Sci. 2021;24(3):1-7. http://doi.org/10.14295/bds.2021.v24i3.2478.

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