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The staining effect of Silver Diamine Fluoride used for caries prevention on sound enamel and enamel caries like lesions: An in-vitro study

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Aim: The aim of this study was to evaluate the staining effect of silver diamine fluoride (SDF) used as a topical agent for caries prevention on both sound and demineralized enamel.

Materials and Methods: Thirty enamel specimens were randomly assigned to three groups. Group I received SDF on sound enamel, Group II received SDF on enamel caries like lesions and Group III received no treatment as the control group. PH cycling model was performed for 7 days . Color assessment was performed for all specimens before treatment and post-treatment after pH cycling.

Results: There was a significant difference in color change values between different groups ($p=0.04$). Group II of SDF on demineralized enamel have a significantly higher mean value (15.71±7.43) of color change than the control group (9.39±4.05) (($p=0.03$). Group I of SDF on sound enamel showed higher mean value (12.14 ±3.4) than the control group but with no statistically significant difference ($p=0.4$).

Conclusion: SDF caused significant dark staining on enamel caries like lesion, while its staining potential on sound enamel was evident to a lesser extent when topically applied as a caries preventive agent.

Keywords: Silver Diamine Fluoride; Caries Prevention; Fluoride; pH-cycling; Dark staining

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Introduction

Early childhood caries (ECC) is one of the most common chronic diseases in children with very high prevalence rates, this stresses on the importance of different caries preventive measures.[1]

The topical application of fluoride containing products is considered the gold standard measure for caries prevention.[2] Silver Diamine Fluoride (SDF) is relatively a newly introduced fluoride product that was approval by the Food and Drug Administration in the United states in 2014 as a desensitizing agent although it has been used for decades in other countries as Japan, China and Brazil.[3] SDF is a solution based on silver, ammonia and fluoride.[4] It has gained attention in the management of dental caries with its 38% concentration as it has antibacterial properties, remineralizing potential on both enamel and dentin and it can cause caries arrest.[5,6]

Although its proven efficacy on carious dentinal lesion but their preventive effect on sound enamel has not been focused on in previous researches.[4]

It is a minimally invasive, simple and cost-effective method, so it can be applied in young and/or uncooperative children as well as it can be applied in community settings.[3,7]

Current methods of prevention like fluoride varnish applications and sealants, although effective to a large extent, but they are costly procedures that cannot be implemented on large populations or vulnerable groups.[8] Besides that the marginal adaptation, wear resistance and long-term retention rates for sealants are crucial for their success as preventive agents.[9] Thus, with increased caries prevalence rates, cost of dental care and inequity among different socioeconomic and ethnic groups, SDF is considered an effective alternative.[10]

The major disadvantage of SDF is its black staining when applied on carious dentinal lesions.[6] However, there are concerns raised about their application on non-cavitated porous enamel lesions on anterior teeth as those lesions might darken as well.[10]

Moreover, the staining potential of SDF on intact sound enamel is another interesting concern that is to be investigated. As, there is no ISO standard for the clinical evaluation of the color of dental hard tissues, using the Commission International del'Eclairage (CIE L*a* b*)color system is considered the most appropriate method for color assessment , as it more closely represents the human perception of color. It is an objective and appropriate tool for color assessment in both in vitro and clinical settings.[11]

Therefore, the aim of this study was to evaluate the staining effect of silver diamine fluoride (SDF) used for caries prevention on sound enamel and enamel caries like lesions under pH cycling conditions.

Materials and methods

This study was approved by the Ethical Committee of the National Research Centre with registration number 13656.

Primary teeth with sound buccal and lingual enamel surfaces indicated for extraction were collected anonymously from the Pediatric dentistry and Dental public health department, Faculty of dentistry, Cairo university.

Crowns were separated by cutting off the roots using diamond disc under coolant.[2] Each tooth was sectioned buccolingually into two halves with the buccal and lingual enamel surfaces being checked under a magnifying lens.[12]

Exclusion criteria included any enamel surfaces with carious lesions, hypomineralized areas, stains, cracks , fracture lines or any flaws.[4]

Each tooth was mounted in a self-cure acrylic resin inside a cylindrical plastic mold its buccal or lingual surface facing upwards.
Enamel specimens were stored in deionized water and were randomly assigned to the different study groups using Random.org.
Study groups:
The specimens were divided into three groups as shown in Table 1.

Table 1: STUDY GROUPS

Groups	Description
Group I	SDF on sound enamel
Group II	SDF on artificial carious enamel lesion
Group III	Control group of deionized water

Specimens of Group II will be immersed first in the demineralizing solution (calcium and phosphate, both 2.0 mmol/L, in 75 mmol/L acetate buffer, pH 4.7; 0.04 µg F/mL, 2.2 mL/mm2) for 36 hours to create an artificial enamel lesion then rinsed with deionized water.[4]

Color assessment:

Baseline color assessment will be performed for all enamel specimens before treatment application.[4]
It was done according to the CIE L*a* b* color system by means of Easyshade dental spectrophotometer (Vita Zahnfabrik, BadSackingen, Germany).[13]
The L* (lightness from black to white), a* (red to green), b* (yellow to blue), h (hue) and C (chroma) will be evaluated 3 times, and the average CIE L*, a*, b*, C*, and h* values were recorded.[14] All color measurements were performed in the center area of each specimen.

Treatment:

For Group I: 38% SDF(SDF, Toothmate , Egypt) solution was applied with a micro-brush on the enamel specimens with sound enamel , the solution was left in contact with the enamel surface for 2 minutes, while for Group II: 38% SDF(SDF, Toothmate , Egypt) solution was applied on enamel specimens with artificial carious lesions as in group 1, while for Group III :No treatment was applied .
All Specimens were rinsed with deionized water.

PH cycling:

PH cycling was performed for 7 days. Each specimen was stored in a separate container. Specimens were immersed in the demineralizing solution (calcium and phosphate, both 2.0 mmol/L, in 75 mmol/L acetate buffer, pH 4.7; 0.04 µg F/mL, 2.2 mL/mm2) for 3 h, followed by deionized water rinse. Then, they were immersed in the remineralizing solution (1.5 mM CaCl2, 0.9 mM NaH2PO4, 0.15 M KCL had a pH of 7.0, 1.1 ml/mm2) for 21 h. The pH cycling was carried for five days and the enamel specimens were left in the remineralizing solution for the last two days.[12]
Post-treatment color assessments were performed after PH cycling to evaluate the color change on enamel specimens.
The change in the color (ΔE) in the groups were calculated using the L*, a*, and b* values, based on the following mathematical equation:

$$\Delta E^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)1/2$$

The ΔL value represents the change in brightness over time , the negative value indicates a decrease in brightness, while the positive value indicates an increase in brightness, and it is obtained by subtracting the post-treatment value from the baseline or pre-treatment one and the same equation is applied for Δa* and Δb*.[13]
The flow chart of the study is shown in Figure 1.

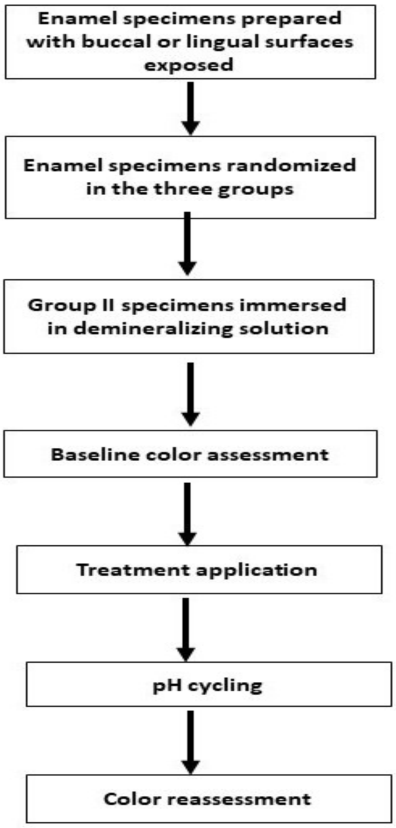

Figure 1: Flow chart of the study.

Sample size calculation:

According to Gadallah et al.[15] the mean value of color change assessment was in SDF group (26.26±10.31) while for the control group of deionized water the mean value was (10.37 ±2.77). Using G power statistical power analysis program (version 3.1.9.7) for sample size determination, a total sample size of 30 (10 in each group) was sufficient to detect a large effect size of 0.62, study power (1-β error) of 0.8 (80%) and a significance level (α error) 0.05 (5%) for the two-sided hypothesis test.

Statistical analysis

Statistical analysis was performed using GNU PSPP Software 1.6.2 (Free Software Foundation, Inc., Boston, USA). Numerical data will be described as mean and standard deviation or as median and range as appropriate. Data will be compared using One-way ANOVA or Kruskal-Wallis test depending on normality.

Results

Numerical data were presented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Data were parametric, so one-way ANOVA followed by Tukey's post hoc test was used to analyze intergroup comparisons. The level of significance was at P≤0.05.Descriptive statistics for color change values were presented in Table 2.

Table2:Descriptive statistics for Color change values

	Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Color change	Group I	10	12.14	3.40	1.07	9.71	14.57	7.15	18.38
	Group II	10	15.71	7.43	2.35	10.39	21.02	5.69	27.87
	Group III	10	9.39	4.05	1.28	6.50	12.29	1.53	16.38
	Total	30	12.41	5.72	1.04	10.28	14.55	1.53	27.87

The highest mean value of the color change ΔE was recorded in Group II of SDF on demineralized enamel (15.71±7.43) while the lowest mean value was recorded for the Group III of the control group (9.39±4.05) as shown in Figure2 .

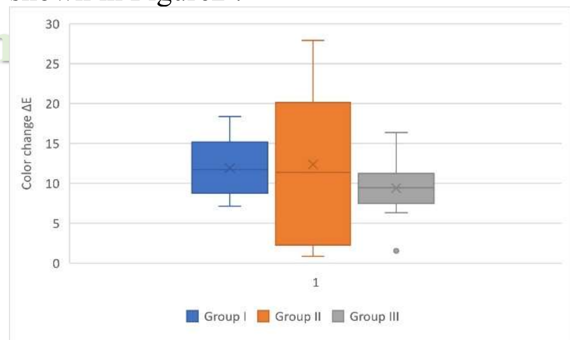

Figure 2: Box plot showing Color change(ΔE) in different groups

Results of intergroup comparisons with one-way ANOVA test showed that there was a significant difference in color change values between different groups (p=0.04). Post hoc pairwise comparisons presented in Table 3 showed Group II of SDF on demineralized enamel have a significantly higher value of color change than the control group (p=0.03) while there was no statistical significant difference between group I of SDF on sound enamel compared to group III as control (p=0.4) and also between group I of SDF on sound enamel compared to group II of SDF on demineralized enamel (p=0.3). These color changes were shown in Figure 3 of the samples' images.

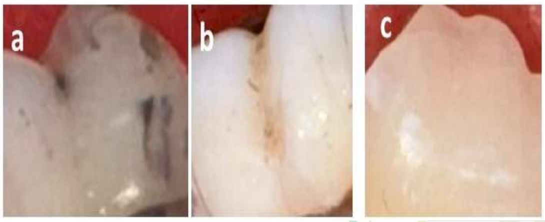

Figure 3: Different group samples. a: Group II demineralized enamel sample showing marked dark staining, b: Group I sound enamel sample showed a lesser degree of staining, c: Group III control enamel sample with normal appearance

Table 3 Tukey's Post hoc analysis for Color change ΔE

	(J) Family	(J) Family	Mean Difference (I - J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Group I	Group II	-3.56	2.36	.301	-9.40	2.28
		Group III	2.75	2.36	.482	-3.09	8.59
	Group II	Group I	3.56	2.36	.301	-2.28	9.40
		Group III	6.31	2.36	.032	.47	12.15
	Group III	Group I	-2.75	2.36	.482	-8.59	3.09
		Group II	-6.31	2.36	.032	-12.15	-.47

Discussion

Silver diamine fluoride has been proven as a minimally invasive and successful technique for the treatment of carious dentinal lesions with reported black staining as their major disadvantage, however, there use as a preventive and remineralizing agent on sound and early enamel carious lesions has not been thoroughly investigated.

The presence of both silver and fluoride ions had shown a synergistic effect in arresting dentinal carious lesions where silver ions have an antibacterial effect by inhibiting the biofilm growth, whereas fluoride has a remineralizing ability by enhancing mineral formation and decreasing its solubility against acidic attacks.[16]

The effect of both ions in caries arrest together with cost-effectiveness, eco-friendliness, moreover the simplicity, less technique sensitivity and the annual application had encouraged some investigators to study the effect of different topical silver and fluoride agents as silver fluoride, silver diamine fluoride with or without potassium iodide for caries prevention on sound or demineralized enamel, however most of them did not evaluate the staining potential of these agents.[4,12,17]

The reported tooth discoloration of SDF is the primary drawback and cause of low parental acceptance rates in its application specifically on anterior teeth.[18–20] Trying to overcome this disadvantage, the after application of potassium iodide solutions was suggested, however, this black discoloration did not completely vanish, but it was slightly diminished.[13–15] This staining potential remains the biggest obstacle in SDF application in caries arrest and prevention.

SDF reacts with hydroxyapatite, and a layer of silver phosphate and silver sulphide precipitates on carious dentin and it is mainly responsible for the black staining [11]

Silver ions penetrate enamel up to a depth of 25 microns enhancing the resistance of carious dentin to acids and enzymatic breakdowns while fluoride ions enhance the remineralization by formation of fluorapatite crystals, SDF application enhances local availability of fluoride ions by two to three folds when compared to other topical fluorides.[21]

The effect of SDF as an alternative remineralizing agent was recently investigated and shown superior results in an invitro study when applied to early artificial enamel caries lesions as it promoted remineralization and inhibited demineralization in two pH cycling models with and without twice daily fluoride exposure which mimics the daily toothbrushing with fluoride and that reflects the importance of SDF if applied in high caries risk patients with limited fluoride exposure and neglected oral hygiene.[4]

The aim of this study was to evaluate the staining potential of SDF used as a topical fluoride agent for caries prevention on both sound and demineralized enamel.

The use of instrument based devices for evaluation of color change is more precise and reliable rather than naked eye evaluation which is considered subjective.[14] In this study an advanced portable dental spectrophotometer was used for color change evaluation.

This study showed that after SDF application and pH cycling, SDF caused significant dark staining on demineralized enamel compared to the control group. Similarly a previous study showed significant discoloration of SDF on early artificial enamel carious lesions.[4] As for the sound enamel group, there was no significant difference on the color change, however a higher mean value was also recorded when compared to the control group. While a previous study of SDF on sound enamel showed that SDF group had shown a statistically significant difference in color change when compared to the control group of deionized water even after pH cycling.[22] The difference could be attributed to that SDF solutions were left in their study on the enamel surface undisturbed for 60 minutes before performing pH cycling while in our study SDF was only applied for two minutes closely mimicking its clinical application.

The study showed that staining effect of SDF is of a persistent nature and resists repeated acidic challenges, this comes in accordance with previous reports.[4,22]

The color change ΔE of 3.7 units or more is reported to be clinically observed and visible by naked eye while the ΔE of only 1 unit can be distinguishable in laboratory settings.[11,14] The clinical observation of color changes for SDF groups in this study was presented.

Comparisons the between the topical application of 38% SDF versus fluoride varnish in both enamel and dentin caries showed that although 38%SDF is more effective in arresting dentin caries than sodium fluoride varnish for both primary and permanent teeth.[23] However the effectiveness of 38% SDF was similar to fluoride varnish in preventing enamel demineralization of primary teeth.[24]

Taking into consideration the reported staining potential of 38% SDF when applied on demineralized, non-cavitated enamel caries lesions, its topical application for the caries prevention on enamel is not recommended.

One of the limitations of this study that is that pH cycling model does not fully mimic the oral cavity due to the absence of oral microbiome, dental biofilm, and actions of the acquired pellicle.[4] Further studies are required to investigate more silver and fluoride products for caries prevention and evaluate their staining potential in both in vitro and in vivo settings.

Conclusion

The topical application of 38% SDF on early enamel caries lesions showed significant discoloration while its staining potential on sound enamel was evident to a lesser extent.

Funding: This research is self-funded

Data availability: All data required for analysis during this study are included in this published article. Raw data are available on request.

Ethics approval and consent to participate: This in vitro study was approved by the Ethical Committee of the National Research Centre with registration number 13656

Competing interest: The authors declare no conflict of interests

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