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Research Article

Assessment of Remineralizing Effect of Silver Diamine Fluoride (SDF) with Depigmented agents on Demineralized enamel surface of permanent teeth (in Vitro Study)

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Abstract: The current study aimed to compare the effect of the application of 38% Silver diamine fluoride (SDF)on the microhardness of artificially induced enamel surface caries of permanent teeth, with sodium fluoride and de-ionized water by Vickers micro-hardness, and scanning electron microscope (SEM), also testing whether the depigmented agent added to 38% silver diamine fluoride affecting the microhardness of artificially induced caries. Materials and methods: The samples consisted of 45 sound maxillary first premolars; the sample was divided randomly into five groups after they were subjected to a freshly prepared demineralizing agent for 4 successive days, and each group consisted of 9 teeth. Group1: demineralization then treated with 38% of SDF with potassium iodide (kl) for 1 hour, Croup2: demineralization then treated with 38% of SDF with glutathione (20% GSH) for 1 hour, Group3: demineralization then treated with 38% of SDF alone for 1 hour, Group4: demineralization then treated with 5% of sodium fluoride for 1 hour (positive control), Group5: demineralization then socked in de-ionized water for the rest of the experiment (negative control). Result: There were statistical differences among all groups after treatment with a remineralizing agent. The microhardness value shows statistically significant differences in which the highest increase was in the SDF alone group, followed by silver diamine fluoride with potassium iodide, silver diamine fluoride with glutathione, and sodium fluoride, respectively, and finally, the deionized water group, which has the lowest means of microhardness. Conclusion: SDF material is effective in arresting initial enamel caries of permanent teeth.

Keywords: Enamel, Silver Diamine Fluoride, Glutathione, Microhardness, Potassium Iodide

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INTRODUCTION

Over the past 20 years, the high frequency of dental caries and rising healthcare costs have created a serious public health issue on a global scale. Additionally, treating dental caries in young, timid, uncooperative children, those with limited access to dental care, or those with financial constraints can be difficult because dental caries in children that are left untreated can result in discomfort, infections, and expensive ER visits and/or hospital stays. Furthermore, it appears that existing approaches to early caries prevention treatment do not effectively halt caries development.(1)

The minimally invasive method of treating dental caries has come to recognize the need to remineralize dental caries lesions and prevent demineralization at its earliest stage.(2) However, high-risk individuals frequently cannot avoid developing caries lesions using current approaches to early preventative therapy (3), which implies the need for creative and alternative prophylactic methods that are less invasive and traumatizing for kids to prevent and control dental caries.(4)

The use of silver diamine fluoride (SDF) to stop caries in dental caries lesions has grown quickly. (5) Its usage as an agent for caries prevention has received less attention, however, it is regarded as a straightforward and affordable strategy that doesn't require complicated training for the healthcare provider or patient involvement. In areas with a lack of resources, this strategy may be quite helpful as an alternative to more expensive preventive measures.(6)

A topical solution called silver diamine fluoride ($Ag(NH_3)_2F$) is made of silver, ammonia, and fluoride. It is an approach for managing caries that is risk-free, efficient, effective, noninvasive, and economical.(7) Fluoride's ability to promote remineralization and silver's antimicrobial qualities work together to stop the spread of dental caries lesions and stop it in its tracks. (7,8) Few studies have highlighted its potential use in avoiding the development of caries lesions, whereas many randomized clinical trials support its usage solely for the treatment of dental caries.(2,6)

The permanent dark staining of the lesion where silver diamine fluoride is administered is the most important non-medical side effect of silver diamine fluoride. This is a significant obstacle to certain patients' and parents' adoption of silver diamine fluoride as a treatment option (depending on the appearance of the caries lesions).(9)
It has been reported and suggested that potassium iodide (KI), which interacts with free silver ions to cause a yellow silver iodide precipitate, can diminish SDF's dark staining. The usefulness of potassium iodide in avoiding the staining effect of SDF without affecting its preventive and arresting effect on dental caries. (10) Glutathione (GSH), a tri-peptide biomolecule, is considered the ideal option for adsorption with silver. (11)

This in vitro study was conducted to compare the remineralizing effect of SDF with depigmented agents (potassium iodide, glutathione) and sodium fluoride 5% (NaF) on the micro-hardness of the enamel surface of maxillary permanent first premolar after artificial carious lesion production. The null hypothesis was that there was no difference between the effects of depigmented agents (potassium iodide, glutathione) with 38% silver diamine fluoride and sodium fluoride NaF 5% on the enamel surface micro-hardness.

MATERIALS AND METHODS

Sample preparation

The sample size was measured by Using G power 3.1.9.7 (Program written by Franz-Faul, Universitatit Kiel, Germany) with the power of study=80%, alpha error of probability=0.05, the correlation between time points is 0.5, and effect size of F is 0.45, with 5 groups and 3-time points, with all these conditions the definite sample size is 9 samples for each group. [12]

The sample consists of 45 sound maxillary first permanent premolars collected from a private dental clinic in Erbil City. All teeth were cleaned with a cumine scaler, prophylaxis was performed using a rubber cup and non-fluoridated pumice, and the teeth were then preserved in a 0.1% thymol solution for two weeks. [13] Each tooth's crown is sectioned from its root at the cementoenamel junction and then longitudinally sectioned with a diamond disk into 0.5 mm in thickness [14], which is then measured using a digital caliper. The tooth is then embedded in an acrylic mold that is surrounded by a (16 mm diameter x 14 mm depth) plastic ring. [15] All enamel surfaces of samples are then covered with adhesive tape, except the middle circular 6 mm x 6 mm in diameter, which appears to polish it. Sof-Lex Disks were used to gently polish the buccal surfaces (3M ESPE, USA). [16] After polishing the enamel surfaces of all samples, the adhesive tape was removed to prevent an effect on the colorimeter test. As in Figure 1.

Figure (1): Teeth poured into the acrylic mold with a plastic ring.

Demineralization process

These mixtures created the demineralizing solution: acetic acid with a concentration of 0.05 Mole, 2.22 mM calcium chloride, and sodium dihydrogen orthophosphate dehydrate (NaH_2PO_4), then, the pH was lowered to 4.4 by adding 1M potassium hydroxide to the mixture. To produce artificial caries-like lesions on the enamel surface, each specimen was immersed separately in the demineralizing solution for four days in succession (96 hours).(17) It was then meticulously cleaned before being placed in a storage container filled with deionized water.

Sample Grouping

The sample was divided randomly into five groups after they were subjected to a freshly prepared demineralizing agent, each group consisted of 9 teeth.

Group 1: demineralization was then treated with 38% SDF with potassium iodide (kl) for 1 hour.

Group 2: demineralization was then treated with 38% SDF with glutathione (20% GSH) for 1 hour.

Group 3: demineralization was then treated with 38% of SDF alone for 1 hour.

Group 4: demineralization was then treated with 5% sodium fluoride for 1 hour (positive control).

Group 5: demineralization was then soaked in de-ionized water for the rest of the experiment (negative control).

All solutions were left on the enamel surface undisturbed for 60 min then rubbed off using sterile cotton swabs. (18)

Surface microhardness assessment

Surface microhardness was measured on 45 teeth, nine from each treatment group, at baseline (sound enamel surface), following demineralization, and subsequently after remineralization. A computerized Vickers microhardness tester with a diamond indenter was used to take the measurements. By applying 500-gram stress for 30 seconds in a vertical direction to the enamel surface, measurements were taken. The same calibrated machine and examiner were used for all of the measurements. The hardness value for each specimen was determined by taking the mean of three indentations from each reading.(19)

Statistical analysis

Version 21 of SPSS was used to analyze the data. The Shapiro-Wilk test was used to determine the normality of all the data ($p > 0.05$). Repeated repetition to compare the means of enamel microhardness across the five groups, analysis of variance (ANOVA) was utilized. Bonferroni post hoc analysis (LSD) was employed to confirm that there were differences between the groups. P values lower than 0.05 were regarded as significant levels.

RESULTS

A descriptive and statistical test of microhardness among phases was illustrated in a table (1) It focuses on comparing baseline tooth enamel microhardness throughout research groups. The baseline enamel microhardness of the study groups did not differ in a statistically significant way (p= 0.0.054). Additionally, this table displayed a comparison of study groups based on the microhardness of teeth's demineralized enamel. Although microhardness in the demineralization phase was found to be higher in SDF followed by de-ionized water and SDF+KI while the lowest in SDF+GSH and NaF respectively but with no significant difference, Determining the demineralization of enamel microhardness between the study groups did not reveal any statistically significant differences (p= 0.125).

Table 1: Descriptive and statistical test of microhardness among phases

Groups		Baseline	Demineralization	Remineralization
SDF+KI	Min.	290.500	200.300	276.900
	Max.	347.500	244.300	327.800
	Mean	325.767	223.100	300.433
	±SD	18.995	14.300	19.151
SDF+GSH	Min.	296.400	214.700	278.600
	Max.	346.200	232.800	318.300
	Mean	315.878	223.024	297.733
	±SD	17.368	6.039	14.534
SDF	Min.	304.500	213.400	296.300
	Max.	354.800	248.600	334.800
	Mean	331.544	231.962	308.478
	±SD	14.696	10.092	11.742
NaF	Min.	293.100	196.900	240.100
	Max.	346.200	243.500	289.300
	Mean	323.589	215.820	268.600
	±SD	15.153	12.979	18.279
De-ionized water	Min.	297.500	193.180	191.260
	Max.	380.000	255.500	237.900
	Mean	346.722	228.529	219.512
	±SD	26.657	19.313	16.123
F		2.55	1.926	45.743
P value		0.054^	0.125^	0.000*

In the Remineralization phase, the SDF group has a higher value in microhardness followed by SDF+KI and SDF+GSH respectively while lower in NaF and de-ionized water respectively with a significant difference between groups and larger variability in which the p-value was <0.05.

Table (2) shows descriptive and statistical tests of microhardness among groups, when comparing the change of microhardness between phases within the same group, all findings appear the microhardness is decreased in the demineralization phase then it will incline again with significant change (p<0.05) in each group furthermore.

A post hoc test (LSD) was performed to confirm the differences that occurred between groups and phases when comparing demineralization and remineralization as shown in table (3), this table showed the mean differences (phase I –phase J) of change of microhardness.

Table (2): Descriptive and statistical test of microhardness among groups.

Groups		**Baseline**	**Demineralization**	**Remineralization**	**F**	**P value**
SDF+KI	Min.	290.500	200.300	276.900	140.064	0.000*
	Max.	347.500	244.300	327.800		
	Mean	325.767	223.100	300.433		
	±SD	18.995	14.300	19.151		
SDF+GS	Min.	296.400	214.700	278.600	125.633	0.000*
	Max.	346.200	232.800	318.300		
	Mean	315.878	223.024	297.733		
	±SD	17.368	6.039	14.534		
SDF	Min.	304.500	213.400	296.300	135.377	0.000*
	Max.	354.800	248.600	334.800		
	Mean	331.544	231.962	308.478		
	±SD	14.696	10.092	11.742		
NaF	Min.	293.100	196.900	240.100	103.540	0.000*
	Max.	346.200	243.500	289.300		
	Mean	323.589	215.820	268.600		
	±SD	15.153	12.979	18.279		
De-ioni water	Min.	297.500	193.180	191.260	168.262	0.000*
	Max.	380.000	255.500	237.900		
	Mean	346.722	228.529	219.512		
	±SD	26.657	19.313	16.123		

*=significant at p<0.05.

Table (3): Post hoc test (LSD) Comparisons of MH among phases by groups

Pairwise Comparisons of MH among phases by groups using Bonferroni posthoc test.

Groups	(I) Phases	(J) Phases	Mean Difference (I-J)	P value
SDF+KI	Base-line	Demin.	1020.667	**0.000***
		Remin.	250.333	**0.002***
	Demin.	Remin.	-770.333	**0.000***
SDF+GSH	Base-line	Demin.	920.853	**0.000***
		Remin.	180.144	**0.035***
	Demin.	Remin.	-740.709	**0.000***
SDF	Base-line	Demin.	990.582	**0.000***
		Remin.	230.067	**0.005***
	Demin.	Remin.	-760.516	**0.000***
NaF	Base-line	Demin.	1070.769	**0.000***
		Remin.	540.989	**0.000***
	Demin.	Remin.	-520.780	**0.000***
De-ionized wate	Base-line	Demin.	1180.193	**0.000***
		Remin.	1270.210	**0.000***
	Demin.	Remin.	90.017	0.221^

*=significant at p<0.05.

All results in each group find that each phase is statistically significant (p<0.05) when compared with another one except in the de-ionized water group when compared the demineralization phase with remineralization, the result was not statistically significant (p =0.221). To confirm the differences that occurred between groups during the remineralization phase Post hoc test (LSD) was performed as shown in table (4), this table showed the mean differences (phase I –phase J) of change of microhardness. Most results were found to be statistically significant in (p <0.05) except when comparing SDF+KI with SDF+GSH the result was not statistically significant (p=1.000). Also, the table shows compression between the SDF+KI and with SDF alone group, the finding was not statistically significant at p=1. 000. In addition, in the SDF+GSH group when compared with the SDF alone group, these findings are not statistically significant (p=1.000).

Table (4): (Pairwise Comparisons) MH among groups in the remineralization Phase using Bonferroni posthoc test.

(I)groups	(J)groups	Mean different(I-J)	P-value
SDF+KI	SDF+GSH	2.700	1.000^
	SDF	-8.044	1.000^
	NaF	31.833	0.002*
	Distal water	80.921	0.000*
SDF+GSH	SDF	-10.744	1.000^
	NaF	29.133	0.005*
	De-ionized w	78.221	0.000*
SDF	NaF	39.878	0.000*
	De-ionized w	88.966	0.000*
NaF	De-ionized w	49.088	0.000*

DISCUSSION

The present in vitro study investigates the remineralizing effect of silver diamine fluoride, SDF with a depigmented agent, and compares it with fluoride varnish on the demineralized enamel surface.

In this study, the enamel surface was artificially demineralized. The reason for selecting artificially demineralized enamel was to assess the staining property of SDF when used as a preventive agent in arresting incipient carious lesions.

To lessen the inherent variation in enamel surface between the samples, which may react differently to acidic dissolution, polished tooth samples were employed in this investigation. [20] Specimens were sectioned from their root to facilitate handling after that mounted in an acrylic mold and also grounded and polished before microhardness measurement. This preparation of the samples was performed because any tilting or non-flat surface might produce a very long indentation and hence a lesser hardness value. Consequently, the surface of a flat specimen was to demonstrate a mineral increase or loss, and various strategies were employed. Surface microhardness analyses, which evaluate the demineralization and remineralization changes that have taken place in enamel, are the most often utilized ones. SMH assessments can be measured quickly, easily, and without causing any damage. Because of its mechanism of action, which depends on determining the resistance of materials' surfaces to plastic deformation from a reliable source, SMH evaluations are a useful choice for assessing mineral changes. This makes it possible to collect measurements from the same object throughout time, reducing experimental variation. [21]

The previous Iraqi study provided successful results when using the microhardness test. [22] The flattest areas of the enamel surface were indented to guarantee the precision of the measurements. The microhardness tester and connected microscope were both magnified 50 times to find the testing surface's flattest spot.

The study's test results showed that there were differences between the tested groups at the baseline and demineralization phases, but these differences were not statistically significant. However, after the addition of the test materials, there was a highly significant difference between the tested groups.

Furthermore, the Multiple Analysis Range Test for test groups illustrated that the silver diamine fluoride group had a minimum reduction in the microhardness mean value followed by silver diamine fluoride with kl, SDF with GSH, the group using fluoride varnish, and the deionized water control group saw the biggest decreases in the enamel's surface microhardness, respectively, this results agreed with Burgess and Vaghela [23], whose were discussing the safety of silver diamine fluoride, the prevention rate for the development of new lesions with silver diamine fluoride was

70.3%, compared to 55.7% with topical fluoride or fluoride varnish. This indicates that silver diamine fluoride will more effectively protect the enamel of permanent dentition than fluoride varnish.

Even though there are no statistical differences between SDF alone, SDF+ kl, and SDF +GSH when comparing the mean of microhardness value among groups the SDF alone group stillthe group which has a higher value of microhardness that means using a depigmented agent may affect caries arresting effect of SDF in a minor way, this result disagreed with the study done by Sorkandi *et al.* [24] that conclude Based on the vicker microhardness test (VHN) results of the chemical model, SDF appears to be a successful topical treatment for the prevention of enamel caries due to the excellent distribution of high concentrations of fluoride ion to the sound enamel surface, in light of this, it can be assumed that fluoride and silver have complementary effects.

Also, the results from the chemical model show that the KI application did not impair the anti-caries efficacy of SDF. That agreed with a study done by Sorkandi *et al.* 2022 [25] which concluded that the SDF might provide an alternative biological method for remineralizing early enamel carious lesions is also controversial. In the context of additional twice-daily fluoride treatments, SDF SDF and KI seem to be equally effective at encouraging the remineralization of early enamel carious lesions. When paired with extra twice-daily fluoride administration, KI application after SDF significantly lowered the dark staining and did not hinder the promotion of remineralization of enamel carious lesions. Meanwhile, the Post hoc test of SDF+GSH groups among phases shows that this group has the least effect on the remineralization of enamel. This may be due to the glutathione material, which decreases the effect of the silver component of SDF by binding with it.[26]

The averages, standard deviations, maximum and minimum values at baseline, demineralization, and remineralization are among the descriptive data provided in the current study. In comparison to the deionized water control group, the preventive effect of silver diamine fluoride was superior to that of silver diamine fluoride with potassium iodide, silver diamine fluoride with glutathione, and fluoride varnish, all of which helped to preserve the microhardness of the enamel surface of permanent teeth. This was in agreement with Shah *et al.* [27] findings, which showed that silver diamine fluoride significantly had a higher fluoride content in the enamel six months after application than fluoride varnish and acidulated phosphate gel. They also noted that, even if the difference was not statistically significant, silver diamine fluoride had a much lower rate of new carious lesions developing than fluoride varnish and acidulated phosphate fluoride. Agreed to a different study, using SDF as a remineralizing agent will greatly increase the microhardness of the enamel surface and be successful in remineralizing the enamel of teeth with early-stage caries. [28] Silver and fluoride ions applied topically may have synergistic remineralizing action and enhance the mineral density of dentine and demineralized enamel. [29] Additionally, numerous in vitro and in vivo research that examined the effectiveness of SDF and fluoride varnish for halting tooth caries came to the same conclusion. [30] Also, in agreement with a study that shows that fluoride and silver ions both demonstrated synergistic effects in halting active dentinal carious lesions, according to a study by

Zhao *et al.* 2018 [20] examined the mechanism of action of SDF as a bactericidal agent that could stop demineralization and encourage remineralization of enamel and dentin. The newly formed crystals were more resistant to acid attack due to the production of silver phosphate and insoluble metallic silver salts by the interaction of silver ions, which would increase the surface hardness of the tooth structure. [31]
Further, a longitudinal study needs to evaluate the remineralizing effect of SDF material.

CONCLUSIONS

Silver diamine fluoride material was effective in enhancing the remineralization of the initial enamel caries, this remineralizing potential was revealed by the increase in enamel microhardness values

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript

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تقييم تأثير إعادة التمعدن لفلوريد ديامين الفضة مع عامل منزوع التصبغ على سطح المينا المنزوع المعادن من الأسنان الدائمة (دراسة مختبرية)

فاطمة ثائر, الحان احمد

الملخص

الأهداف: هدفت الدراسة الحالية إلى مقارنة تأثير تطبيق 38 ٪ من الفضة ديامين فوريد (سدف) على ميكروهاردنيس من التسوس سطح المينا المستحث صناعيا من الأسنان الدائمة ، مع فلوريد الصوديوم والمياه دي المتأينة من قبل فيكرز صلابة الصغرى ، والمسح الضوئي المجهر الإلكتروني (سيم) ، وأيضا اختبار ما إذا كان عامل ديبيجمينتد إضافة إلى 38 ٪ فلوريد ديامين الفضة التي تؤثر على ميكروهاردنيس من التسوس المستحث صناعيا. **المواد وطرائق العمل**: تتكون العينات من 45 صوت الضواحك الأولى الفك العلوي; تم تقسيم العينة بشكل عشوائي إلى خمس مجموعات بعد أن تعرضوا لعامل إزالة المعادن الطازج لمدة 4 أيام متتالية ، وتألفت كل مجموعة من 9 أسنان. المجموعة 1: إزالة المعادن ثم تعامل مع 38 ٪ من سدف مع يوديد البوتاسيوم (كل) لمدة 1 ساعة ، كروب2: إزالة المعادن ثم تعامل مع 38 ٪ من سدف مع الجلوتاثيون (20 ٪ غش) لمدة 1 ساعة ، المجموعة 3: إزالة المعادن ثم تعامل مع 38 ٪ من سدف وحدها لمدة 1 ساعة ، المجموعة 4: إزالة المعادن ثم تعامل مع 5 ٪ من فلوريد الصوديوم لمدة 1 ساعة (السيطرة الإيجابية) ، المجموعة 5: إزالة المعادن ثم مضروب في المياه. **النتائج**: كانت هناك اختلافات إحصائية بين جميع المجموعات بعد العلاج بعامل إعادة التمعدن. تظهر قيمة الصلابة الدقيقة فروق ذات دلالة إحصائية حيث كانت أعلى زيادة في مجموعة قسد وحدها ، تليها فلوريد ديامين الفضة مع يوديد البوتاسيوم ، وفلوريد ديامين الفضة مع الجلوتاثيون ، وفلوريد الصوديوم ، على التوالي ، وأخيرا ، مجموعة الماء منزوع الأيونات ، والتي لديها أدنى وسائل الصلابة الدقيقة. **الاستنتاجات**: مادة سدف فعالة في القبض على تسوس المينا الأولي للأسنان الدائمة.