

The Effect of Silver Diamine Fluoride and Fluoride Varnish on Microhardness of Primary Teeth Enamel (An In Vitro Study)

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الخلاصة

الأهداف: إن الهدف من هذه الدراسة هو مقارنة المواد الفعالة لإعادة بناء العناصر الداخلة في تركيب أسطح الأسنان متمثلة ب الفلوريد فلوريد ثنائي أمين الفضة ثم تقييم تأثيرهما على صلادة أسطح الميناء للأسنان اللبنية في الدراسة التي تمت مختبرياً. **المواد وطرائق العمل:** عدد الأسنان الكلي للعينه (١٥٠) من الأسنان الأمامية اللبنية. تم تحضير عينات اسطح الميناء و تقسيمها عشوائيا إلى ثلاث مجموعات: مجموعة الفلوريد فلوريد ثنائي أمين الفضة (٥٠ عينة)، بالإضافة إلى مجموعة السيطرة المتمثلة بالماء منزوع الأيونات (٥٠ عينة) و تم تعريض المجموعات الثلاث لدورة الاس الهيدروجيني. تم قياس صلادة الأسنان قبل وبعد تعريضها لدورة الاس الهيدروجيني باستخدام جهاز قياس الصلادة (المايكرو فكر). **النتائج:** كان هناك اختلاف ذات دلالة إحصائية عالية بين مجموعات الدراسة بعد دورة الاس الهيدروجيني ، و كان هناك انخفاض في صلادة الأسطح في جميع المجموعات بسبب فقدان العناصر المعدنية و لكن الانخفاض الأدنى في صلادة السطح كان في مجموعة الفضة ثنائي أمين الفلوريد متبوعا ب مجموعة الفلوريد فلوريد ثنائي أمين الفضة. **الاستنتاجات:** من خلال الدراسة تبين ان مجموعة فلوريد ثنائي أمين الفلوريد لها تأثير واضح في الحفاظ على صلادة سطح الميناء و مقاومة فقدان العناصر المكونة لتركيب الأسنان

ABSTRACT

Aims: This study aims to compare and evaluate the effect of two remineralizing agents: fluoride varnish and silver diamine fluoride solution on the surface microhardness of enamel of primary teeth. **Materials and methods:** A total of (150) primary anterior teeth were used in the study. Enamel blocks were prepared and divided into three groups: Fluoride varnish n(50), silver diamine fluoride n(50) and the control group of deionized water n(50), then introduced into PH cycle. Microhardness of enamel blocks was measured using Vickers microhardness tester machine (OTTO Wolpert-WERKE GMBH) before and after the PH cycle. **Results:** There were highly statistically significant differences among study groups after PH cycle and there were a decreasing in surface microhardness in all groups due to the demineralization, but the least reduction in surface microhardness belonged to silver diamine fluoride group followed by fluoride varnish group. **Conclusions:** Silver diamine fluoride was significantly better than fluoride varnish in preserving enamel's hardness and resistant to demineralization.

Key words: Enamel demineralization, Silver di amine fluoride, Fluoride varnish, Microhardness.

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INTRODUCTION

Early childhood caries is still one of the most prevalent, rapidly progressing microbial disease affecting children worldwide. It is influenced by sugar-rich diet and poor oral hygiene or insufficient dental plaque removal that is not necessarily related to bottle feeding. It is resulted in dental destruction, pain also place the permanent dentition at risk for developing the dental caries. In addition, it can negatively influence the quality of life of children and their caregivers⁽¹⁾. The enamel of primary dentition is only half as thick as that of permanent dentition^(2,3). It also has a higher organic and lower mineral contents, making it more liable to carious attack than the enamel of permanent dentition^(4,3). Recent treatments focus on the application of remineralizing agents to incipient the caries to control demineralization and promote remineralization. Remineralizing agents make a super saturated environment around the decayed lesion, thus, it will prevent mineral loss and concentrate the phosphate and calcium ions in the vacant areas⁽⁵⁾. Fluoride is the most effective measure for the prevention of demineralization which can be used either topically or systemically⁽⁶⁾. Fluoride also assists to speed remineralization as well as disrupts the acid production in already erupted teeth of both children and adults^(7,8). Fluoride varnish (professional use, typically, 22,600 ppm F) was effective in preventing caries in both primary and permanent teeth^(9,10). Recently published

multiple systematic reviews point out that silver diamine fluoride (SDF) application could optimally arrest dental carious lesions in children⁽¹¹⁻¹⁴⁾, and treatment using SDF has been shown to be 89% more effective than other active treatments or placebo in arresting dental caries of primary teeth⁽¹⁴⁾. SDF application could be widely recommended and promoted as an alternative preventive approach for the conventional invasive caries management, especially among children patients who are too young to receive dental care, those with special care needs, or those with difficulty in attaining and affording the conventional dental care⁽¹⁵⁾.

The present study aims to compare and evaluate the effect of two remineralizing agents: fluoride varnish and silver diamine fluoride solution on the surface microhardness of enamel of primary teeth in an vitro study.

MATERIALS AND METHODS

Sample collection:

The sample of this study consisted of 150 newly extracted human primary anterior teeth with specific criteria: Intact upper and lower primary anterior teeth are collected, being free of caries, having no fillings, no developmental anomalies, no enamel hypoplasia, no cracks, wears or fractures. Also, enamel surface should be unaffected by a chemical agent as bleaching agent or acid etching.

Teeth Samples Preparation:

The extracted teeth were cleaned, washed with deionized water and kept in a thymol solution 0.1% to keep the samples wet and avoid the growth of bacteria and fungi on teeth surfaces^(16,18). Teeth had been examined under stereomicroscope (UNION/JAPAN). Only the intact teeth had been included in the study and about fifteen teeth had been excluded.

Teeth had been cleaned, polished with non-fluoridated pumice and rubber cups, the remaining roots had been cut 2mm below the level of cemento enamel junction using a straight diamond bur with copious water irrigation in order not to harm the crowns. All teeth are thoroughly washed with deionized

water and kept in a 0.1 % thymol solution until being mounted in a chemical cured resin in plastic rings. Plastic rings had been cut and prepared in such a way that the upper and lower sides had been flat and parallel to each other (16mm diameter ×14mm depth). Each ring had one primary tooth that was fixed at the top surface of the ring in the center with the labial surface of the tooth exposed so that enamel block of 4x4mm window was obtained⁽¹⁹⁾. The rings with the exposed surfaces of teeth were polished one by one with a fin grit silicon carbide paper (600-, 800-, and 2400- grit) as in Figure (1). Lastly, all samples were washed with deionized water and kept till the starting of PHcycle.



Figure (1):The crowns were mounted in cylindrical plastic tubes (16mm diameter ×14mm depth) with cold cure acrylic resin with the outer buccal enamel surface exposed

Materials:

The materials used in this study are: Fluoride Varnish / Ftor-Lux (sodium fluoride 1%, Calcium fluoride 4%, Aminofluoride 0.5%) Techno Dent/Russia, and Silver Di-Amine Fluoride solution / CARIES STOP 30% / biodinamica/ Brasil / PH 8.5.

The Experimental Design of the Study:

The total number of teeth samples were randomly divided into three main groups, each one contains (50) teeth samples as follows:

Group 1(Fluoride Varnish): The teeth were exposed to fluoride varnish for 24 hrs and stored in deionized water, then the layer of

varnish was removed with ascalpel blade and cotton soaked with acetone. Being careful not to scratch the surfaces, samples washed with deionized water for 1 min then introduced to PH cycle⁽¹⁹⁾.

Group 2 (Silver Diamine Fluoride): the teeth surfaces were exposed to silver diamine

fluoride solution with a small brush for 2 min then washed with deionized water for 30 sec and lightly dried with absorbent papers, then introduced to PH cycle⁽¹⁹⁾.

Group 3 (Control): no agent was applied, only washed with deionized water. Experimental Design of the Study seen in Figure (2).

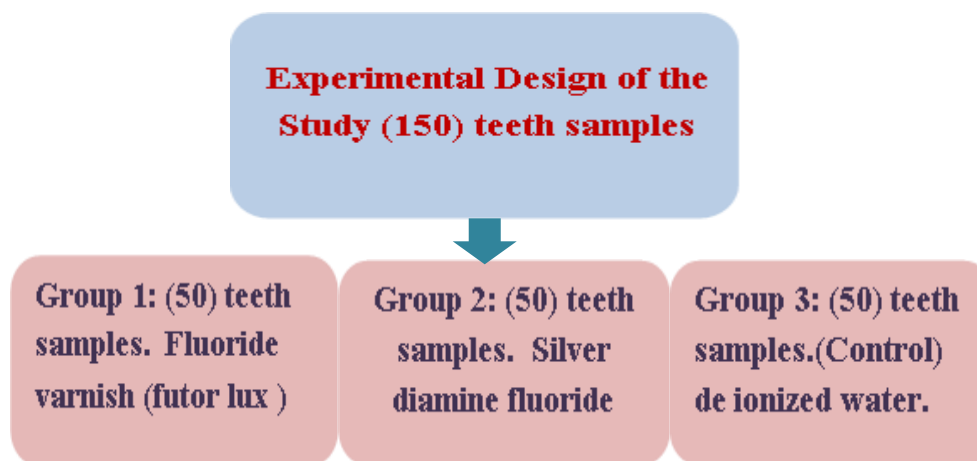


Figure (2):Experimental Design of the Study

Solutions preparation

Chemical materials were imported from College of Chemical Science, College of Dentistry/ Department of basic science and The Main laboratory of the University of Mosul after getting the formal transaction to deal and barrow chemical materials.

According to the laboratory general safety rules, chemical materials had been handled after weighting it by accurate digital balance, then mixing it in one liter of deionized water until it completely dissolved. PH meter had been used to check the PH of the prepared

solutions. Preparation of chemical solution was done at College of Dentistry\Department of Basic Science at the Chemistry laboratory.

1.Demineralization solution: The demineralizing solution consists of CaCl_2 and NaH_2PO_4 both at 2.2 mM, and acetic acid at 0.05 M, pH equal to 4.5, could be adjusted with KOH at 1M , 15 ml/tooth⁽²⁰⁾.

2.Remineralization solution: The remineralizing solution consists of 1.5m M CaCl_2 , 0.9 mM NaHPO_4 , 0.15 mM KCl , pH of 7.0, 15 mL/tooth⁽²⁰⁾.

3. Artificial saliva: Components of artificial saliva are NaCl 0.40, KCl 0.40, CaCl₂.2H₂O 0.79, NaH₂PO₄.2H₂O 0.78, Na₂S₂O₈.H₂O 0.005, CO(NH₂)₂ Urea 0.1, in 1000 ml Distilled water, pH of 7 (concentrations G\L)⁽²¹⁾. The unclear, cloudy liquid of remineralizing solution and artificial saliva had been filtered using filter papers, after that when minerals had been completely stabilized, the PH was measured to be sure that no changes of PH readings will occur days later.

PH-cycling:

The formation of artificial caries occurs by submission of teeth samples to PH cycling. Teeth samples were kept in demineralizing solution PH of 4.5 for 3 hours and in remineralizing solution PH of 7.0 for 20 hours. Teeth samples were washed with deionized water briefly between solutions and kept in artificial saliva for 30 minutes at the end of the demineralization and remineralization process. The teeth were introduced to a total of 10 cycles and the duration of each cycle was one day (24 hours). The demineralizing – remineralizing solutions were changed each day, and the artificial saliva was replaced after every treatment^(22, 23).

Microhardness Assessment:

The mechanical properties of teeth samples were measured using a Vickers

microhardness machine (OTTO Wolpert–WERKE GMBH, V- Tester 2/ Germany) as shown in Figure (3), the machine was invented by Smith and Sandly in 1921 being alternative to Brinell method⁽²⁴⁾. The microhardness numbers were calculated from the length of the indentation on the buccal enamel surface and the indentation length was then determined microscopically (µm) with 70X magnification. Three indentations were made on each sample at the occlusal third of the enamel surface and the mean value was taken for each sample^(25, 26). This test was conducted at the technical institute / Mosul University. The enamel surface sample was subjected to fixed minor load of 500 gm of force to the samples for 15 seconds. The load and time were constant for all samples throughout the study (according to the instruction of the machine). The size of the indent was determined optically by measuring the two diagonals of the square indent as shown in Figure (4 A and B). The Vicker hardness number was calculated basing on the formula⁽²⁷⁾

$$VHN = (kg \text{ mm}^2) = 185.4 \times P \sqrt{d}$$

P= the testing load in grams.

d= the length of the diagonal line across the indentation in microns.



Figure (3): Vickers microhardness tester machine

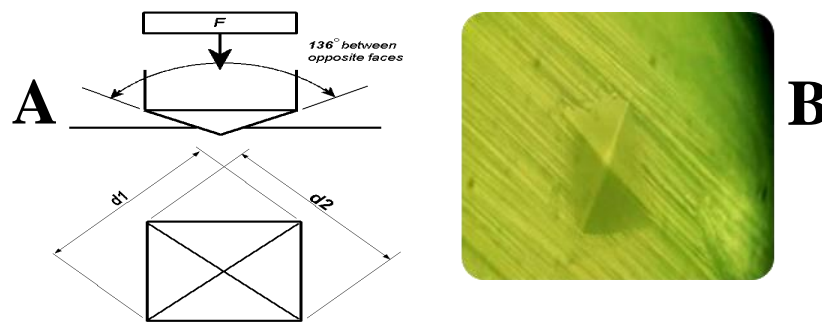


Figure (4): **A**) A schematic illustration of Vickers technique (Vickers microhardness tester machine utilizes a 136° diamond pyramid indenter that forms a square indent on the surface .**B**) An image of a tetra pyramidal indentation in Vickers technique that creates a clear, measurable indentation in the field as diagonals

RESULTS

According to descriptive statistic SPSS version 19, the mean values of microhardness test results were obtained before and after PH cycle explained in Table (1) silver diamine fluoride is better than fluoride varnish and both are beneficial to protect the microhardness of enamel of primary teeth in comparison with the control group of deionized water.

Table (2) ANOVA test (Analysis Of Variance) demonstrating the difference among test groups before and after PH cycle and explained that no significant difference among groups was observed before PH cycle while after the addition of test materials and introduction in to PH cycle, there was a highly significant difference among groups at $p \leq 0.01$, the result among tested groups after PH cycle was further explained at Table (3) Duncan^a Multiple Analysis Range Test. The percentage

of loss of microhardness were calculated for all groups and explained in Figure (5) at which the silver diamine group shows the least reduction in surface microhardness followed

by fluoride varnish and the maximum value of surface microhardness loss belongs to the control group of deionized water where no remineralizing agent was applied.

Table (1): Means, standard deviations and numbers of samples among tested groups before and after PH Cycle.

Variables	Groups	Before PH Cycle	After PH Cycle
Fluoride Varnish	Mean	203.418	182.294
	Std. Deviation	10.536	9.731
	N	50	50
Silver Di Amine Fluoride	Mean	204.764	189.882
	Std. Deviation	9.364	8.897
	N	50	50
De-ionized Water	Mean	207.890	176.289
	Std. Deviation	12.265	9.169
	N	50	50

Table (2): ANOVA test explaining highly significant difference between tested groups after PH cycle at $p \leq 0.01$.

		Sum of Squares	df	Mean Square	F	Sig.
Before PH Cycle	Between Groups	526.442	2	263.221	2.262	.108
	Within Groups	17108.866	147	116.387		
	Total	17635.308	149			
After PH Cycle	Between Groups	4640.627	2	2320.313	26.988	0.000*
	Within Groups	12638.605	147	85.977		
	Total	17279.232	149			

* highly significant difference at $p \leq 0.01$

Table (3) Duncan^a Multiple Analysis Range Test for tested groups after PH Cycle.

GROUPS	N	Subset for alpha = 0.01		
		1	2	3
-De ionized water	50	176.289		
-Fluoride varnish	50		182.294	
-Silver Di amine Fluoride	50			189.882
Sig.		1.000	1.000	1.000

highly significant difference at $p \leq 0.01$

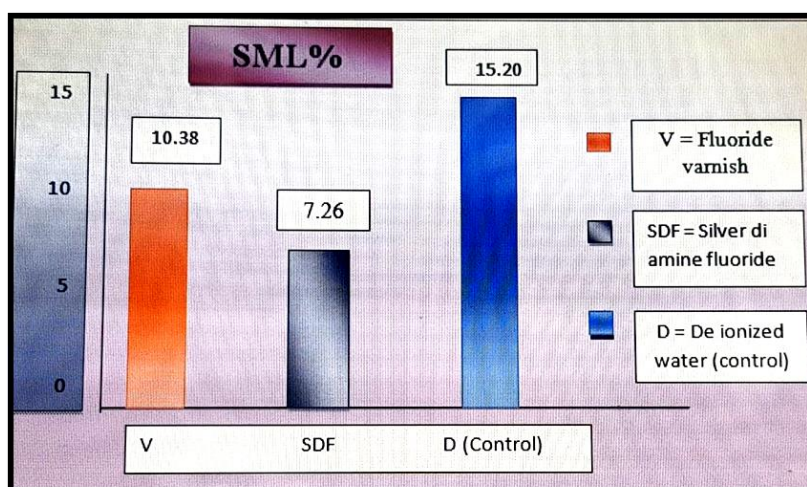


Figure (5): The percentage of surface micro hardness loss for all groups SML% according to equation: $SML\% = \frac{SMH2 - SMH1}{SMH1} \times 100$

SML%: the percentage of microhardness loss.

SMH1: surface microhardness before PH cycle.

SMH2: surface microhardness after PH cycle.

DISCUSSION

Silver diamine fluoride (SDF) a topical agent, has been considered as an effective, equitable, safe, simple and noninvasive caries preventive agent⁽²⁸⁾. It could be applied without the removal of dental caries⁽¹²⁾. Clinical trial declared that SDF is an effective topical agent for preventing and arresting dental caries in primary and permanent dentition⁽²⁹⁾. The use of SDF in dentistry has acquired much attention

globally due to its approval by the US Food and Drug Administration in August 2014⁽³⁰⁾. Table (1) explained the difference in means values before and after PH cycle and showed that silver diamine fluoride was better than fluoride varnish and both were beneficial to protect the microhardness of enamel of primary teeth in comparison with the control group of deionized water. Table (2) demonstrating the difference among test groups before and after the PH cycle and noticed that no significant difference

among groups before PH cycle while after the addition of test materials and introduction in to PH cycle, there was highly significant difference among groups as noticed at Table (3) of Duncan^a Multiple Analysis Range Test for groups after PH Cycle. Figure (5) explained the percentage of loss of microhardness for all study groups which was obtained from the equation:

$$SML\% = \frac{SMH2 - SMH1}{SMH1} \times 100$$

(SML%: The percentage of microhardness loss, SMH1: Surface microhardness before PH cycle and SMH2: Surface microhardness after PH cycle)⁽¹⁹⁾. The percentage of loss of microhardness for fluoride varnish group was less than that of the control group, so both silver diamine fluoride and fluoride varnish could prevent further loss of mineral during PH cycle of artificial the enamel caries while providing additional minerals to protect enamel of primary teeth but silver di amine fluoride was better than fluoride varnish in preventing mineral loss. The result was agreed with Oliveira *et al.*, 2019 who concluded that silver diamine fluoride is better than fluoride varnish and placebo groups in preventing dental caries of entire dentition⁽³¹⁾. Other researchers found that both silver di amine and fluoride varnish act similarly in preventing enamel caries as Mohammadi and Far (2018) who concluded that both silver diamine fluoride and fluoride varnish had high resistance to enamel demineralization compared to control group of deionized water⁽¹⁹⁾, While other researchers had

a different point of view that fluoride varnish was more effective than silver diamine fluoride in reduction of enamel surface demineralization and reduction of lesion depth⁽³²⁾.

The union of fluoride and silver in an alkaline solution has a synergistic effect in arresting dental caries, which makes SDF different from other fluoride agents⁽³³⁾. Additionally, SDF can react with calcium and phosphate ions to form fluoro-hydroxyapatite crystal with reduced solubility, which is considered an important factor for arresting dental caries⁽³⁴⁾. The fluoride penetrates deeper into the tooth structure with SDF as compared with other fluoride solutions, producing a fluoride reservoir in the tooth structure. The fluoride component of contributes remineralization and fluorapatite formation, producing tooth structures which is harder and more caries-resistant⁽³⁵⁾. Furthermore, concentrations of silver ions and fluorine, prevent the growth of cariogenic biofilms in multi-species because SDF is highly effective against the cariogenic biofilm of *Streptococcus mutans*⁽³⁶⁾.

On the other hand, Laboratory studies have revealed that following SDF application an insoluble protective layer of silver phosphate and silver chloride is formed on tooth surfaces and this layer will reduce calcium and phosphorus loss from demineralized enamel and dentin, additional investigation in vivo is necessary to know the actual role of this silver compound⁽³⁷⁾.

CONCLUSIONS

In spite of the fact that both fluoride varnish and silver diamine fluoride solution could protect the enamel surfaces of deciduous teeth but silver diamine fluoride effects were significantly greater than fluoride varnish and further researches were needed to overcome the limitation of black staining associated with SDF treatment of the carious lesion .

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