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EVALUATION OF SILVER DIAMINE FLUORIDE AS NON-INVASIVE TREATMENT ON DENTIN REMINERALIZATION OF CARIOUS PRIMARY MOLARS (AN IN VITRO STUDY)

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ABSTRACT

Objectives: The management of a carious lesion includes complete mechanical removal of the infected, demineralized tooth structure. The present study was conducted to evaluate the effect of SDF on dentin remineralization of carious primary molars in vitro. **Subjects and methods:** A total of 27 carious dentin specimens were used. For Remineralization Test: a total of 27 dentine specimens were used for this test, 18 carious dentine specimens, 9 sound dentine specimens and were divided into three main group (n=9) as follow: Group I: Sound dentine specimens without SDF (Negative control group). Group II: Carious dentine specimens without SDF (positive control group). Group III: Carious dentine specimens with SDF (test group). **Results**: the highest values of Ca/P ratio was statistically significant and recorded for SDF (1.92±0.35), followed by the sound dentine (1.79±0.02). The effect of silver diamine fluoride on carious dentin of primary molars was evaluated including dentin remineralization. **Conclusion**: SDF was able to remineralize the carious primary dentine.

KEYWORDS: Dentin remineralization, Silver diamine fluoride, Energy dispersive x-ray.

INTRODUCTION

Dental caries is an infectious microbiologic lesion of the hard enamel and dentin tissues [(1)]. The management of this lesion depends on the invasiveness of the lesion in enamel and/or dentin and the degree of tissue removal associated with the procedure [(2)]. The conventional protocol for the management of a carious lesion includes complete mechanical removal of the infected, demineralized tooth structure and placement of a restoration [(3)].

However, because the caries process is now understood to be primarily driven by a metabolic process in the dental plaque that leads to demineralization, there is a strong focus on sealing the carious lesion instead of excavating all dentinal caries [(4)]. As a result, conservative caries excavation approaches such as selective caries excavation to soft or solid dentin have emerged [(5)].

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The goal of minimally invasive dentistry is to prevent tooth decay or remove as little tooth structure as feasible [6]. Instead of doing extensive cavity preparation while treating a carious lesion, a minimally invasive technique is indicated to maintain both sound tooth tissue and tissues with remineralization potential [7]. The goal of treating dentinal caries is to remove the outer layer of infected caries and treat the interior damaged dentin based on this notion [8].

Silver diamine fluoride (SDF), is a colourless alkaline solution containing the diamine-silver ion and the fluoride ion. The diamine-silver ion complex consists of two ammonia molecules linked to a silver ion, making it more stable and less oxidising than silver ion [9]. SDF can be a beneficial technique in the minimal-intervention management of dental caries, preventing and arresting cavities in both primary and permanent teeth [10].

The development of silver phosphate precipitate and calcium fluoride, from which fluoride is accessible for remineralization, is attributed with SDF's capacity to stop existing caries [11]. The outmost surface layer of the SDF stopped dentine caries lesion has a considerable increase in microhardness due to an enhanced quantity of calcium and phosphorus[12]. Furthermore, it has a broad antibacterial range of activity against a variety of cariogenic pathogens[13].

Tooth-colored restorations, such as glass ionomer cement (GIC), can hide the black stain left by SDF-treated caries lesions, enhance aesthetics, and increase parental satisfaction with their child's teeth [14]. Because the dentine surface of cavities has been treated with SDF prior to the insertion of the restoration, its remineralization impact on dentin must be investigated. Finally, more study is needed in the area of SDF as a result, the purpose of this study was to see how SDF affected dentin remineralization in carious primary molars in vitro.

SUBJECTS AND METHODS

Study design:

Experimental in vitro controlled study.

Study setting:

The study was carried out in Pedodontics and Oral Health Department, Faculty of Dentistry (Boys, Cairo), Al-Azhar University.

Eligibility criteria for samples selection:

The selection of the involved teeth in this in vitro study were based on specific, and specially designed inclusion and exclusion criteria as the follow: [4]

Inclusion criteria:

Sound primary molars, with carious lesion limited to the outer enamel and No cracks or structural defects.

Exclusion Criteria:

A Non-restorable tooth and tooth with developmental enamel defect.

Sample size:

From the results of a previously published study of Uchil et al [4], a power calculation of sample size indicated that a minimum of 6 teeth per group were required to detect a significant difference between groups, which subsequently increased to 9 per group. The effect size (dz=3.137) and the required sample size were calculated for 95% confidence interval and a power of (0.2315).

Ethical Consideration:

This work was approved by the Ethical Committee of the Faculty of Dental Medicine, Al-Azhar University (Boys, Cairo), with the permission number EC Ref. No. (652/3474).

Sample Grouping For Remineralization Test: A total of 27 dentine specimens were used for this test, 18 carious dentine specimens, 9 sound dentine

specimens and were divided into three main group (n=9) as follow:

- Group I: Sound dentine specimens without SDF (Negative control group).
- Group II: Carious dentine specimens without SDF (positive control group).
- Group III: Carious dentine specimens with SDF (test group).

Intervention:

Sample selection:

- A total of 27 primary molars extracted for normal exfoliation were collected and used in this in vitro study.
- Primary molars without dental cavities or caries localised to the outer enamel were chosen.[4]
- The teeth were kept in 10% formalin for at least 14 days and no more than a month after being collected [4].
- The teeth were checked under a light microscope for fractures and structural problems, and those that had them were eliminated.
- Preparation of samples:
- Two millimetres below the cemento-enamel junction, the roots were severed (CEJ).
- The pulp chambers were cleaned with a big round bur in a slow-speed handpiece, and the pulp was excavated from the root end using a spoon excavator.
- The cleansed pulp chambers were filled and sealed with resin composite (Grandio VOCO GmbH, Germany) to enhance the tooth's resistant form.
- To achieve a uniform at dentin surface perpendicular to the long axis of the teeth, the occlusal enamel was cut using a slow-speed diamond disc under water coolant.
- On a water-cooled lathe, the dentin surfaces were next abraded and smoothed with silicon carbide paper (600 grit), exposing a flat dentin surface and lowering the dentin thickness by 1mm.
- The exposed tooth surfaces were checked under a light microscope to confirm that no enamel remained (Figure 1 a).

Artificial Caries induction:

- Caries was microbiologically induced on the exposed dentin by inoculating Streptococcus mutans.
- The specimens were autoclaved first, then transferred to a cariogenic solution aseptically.
- For every 100 ml of distilled water with a pH of roughly 4.0, the cariogenic solution contained 3.7 g of brain–heart infusion (BHI) broth, 2.0 g of sucrose, 1.0 g of glucose, and 0.5 g of yeast extract.
- The solution was autoclaved at 121°C for 20 minutes before being inoculated with 2 percent S. mutans [4]. (Figure 1 b).
- The teeth were submerged in an acidic cariogenic solution and cultured for 6 weeks at 37°C in a CO2 incubator [4].
- Every 48 hours, the tooth specimens were moved to a container containing a fresh cariogenic solution to offer extra fresh substrate to the bacteria [4].
- The organism's viability was maintained by subculture into new BHI broth every 24 hours.
- The biofilm covering the teeth was removed with gauze when caries induction was confirmed, and the specimens were autoclaved [4].

Evaluation of caries induction:

The presence of caries was confirmed by examining the dentin. The final point of caries induction was defined as a change in dentin colour to yellowish brown and softness felt with a blunt probe **[4].**

SDF application:

Dentine surface of each tooth sample along with group III was treated with 38% SDF solution using a micro-brush for three minutes. Then the surface was rinsed with water for 30-second.

Artificial saliva preparation:

- 1000mL distilled water was mixed with 0.400g natrium chloride, 0.400g potassium chloride, 0.795g calcium chloride monohydrate, 0.69g sodium dihydrogen phosphate, 0.005g sodium sulphide non-anhydrate, and 1.0g urea to make artificial saliva.
- The containers were incubated for 24 hours at 37°C.

Testing procedures:

Evaluation of remineralization: After SDF application and storage in artificial saliva 7 days at 37°c in incubator, nine specimens in each group were subjected to energy dispersive x-ray EDX spectroscopy(Quantax75, Made by Bruker Nano GmbH Germany) to measure minerals content (calcium (Ca), phosphorus (P), and fluoride (F)), Figure 1c.

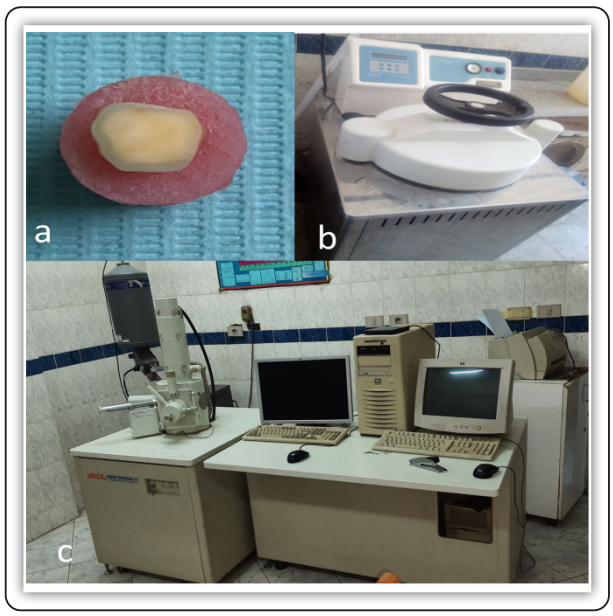

FIG (1) **a)** Tooth sample preparation, **b)** Autoclave used for caries induction, **c)** Energy dispersive x-ray (EDX) spectroscopy.

Statistical analysis

The collected data during the study were tabulated and statistically analyzed using SPSS version 22. The Statistical tests used are Student t-test between the two groups, One-way analysis of variance (ANOVA) test between groups,Chi-square test to compare the numerical values.Level of significant was at p-value < 0. Comparison among the groups was done using Post-Hock's test.

RESULTS

Energy dispersive x-ray (Edx)Analysis (Remineralization):

The statistical analysis of Ca/P ratio (remineralization) test revealed that; there was a statistically significant difference in Ca/P ratio between the studied groups as indicated (p=0.014). Where; the highest (mean ± SD) values of Ca/P ratio was recorded with carious dentine treated with SDF (1.92±0.35), followed by the sound dentine group which recorded Ca/P ratio (1.79±0.02). While, the lowest (mean ± SD) value of Ca/P ratio was recorded with carious dentine group (1.47±0.04).

TABLE (1) Comparison of Ca, P, and F atomic % values of all tested groups.

Variable	Atomic % (Mean ±SD)		
	Ca	P	F
Sound	64.31±0.26	35.68±0.26	0.0±0.0
Carious	59.66±0.68	40.34±0.68	0.0±0.0
SDF	44.06±11.51	22.67±2.02	33.27±13.37

TABLE (2) Comparison of Ca/P values at dentine surfaces of all tested groups.

Variable	Ca/P ratio (Mean ±SD)	f-ratio	p-value
Sound	1.79±0.02^{A}		
Carious	1.47±0.04^{B}	6.1600	0.014*
SDF/caries	1.92±0.35^{A}		

**; The results statistically at p<0.05. upper case letter different significant and same non-significant.*

DISCUSSION

In this study caries induction was conducted by bacteria biofilm to simulate the normal caries process that occurs in the oral cavity and to get caries- affected dentine that simulate the color and texture of natural carious lesion [4,15]. Change in the color of the dentin to yellowish brown and softness caries was confirmed by examining of the dentine by a blunt probe was considered the end point of caries induction in this study as it in agreement with clinical examination [15]. The biofilm covering the teeth was cleaned with a gauze, and the specimens were autoclaved to eliminate the bacterial bias in this study [4].

Prior to the administration of the SDF solution, the carious dentine was conditioned and/or acid etched in the current investigation. This is because the administration of SDF following acid etching improves fluoride uptake in the demineralized dentin while preventing strontium ion uptake, as well as remineralization **[4].**

Dentine remineralization:

The results of the present study showed that the carious dentine samples and the normal dentine samples have no fluoride in their mineral content when studied with EDX. While, the carious dentine samples which treated with SDF have fluoride in their content. This may be because the SDF releases fluoride ions which deposited in the carious dentine[16].

This result agreed with the results of a clinical study conducted by Sinha et al., [11] and confirmed that SDF increased the level of calcium, phosphate, and fluoride ions in caries- affected dentin, with the highest being fluoride ions. As it was postulated that SDF reacts with hydroxy- apatite, forming fluoroapatite and insoluble silver phosphate [17].

Also, according to Mei et al. [18] powder X-ray diffraction (XRD) investigation, SDF treatment of carious tooth structure led in the development of fluorohydroxyapatite, which hardens the soft carious lesion and shows dental remineralization. This showed that in hydroxyapatite crystals, tiny localised fluoride anions replaced hydroxyl anions. This is consistent with the findings of the current investigation, in which fluoride ions replaced hydroxyl anions, resulting in the creation of fluorohydroxyapatite.

Furthermore, Mei et al. [18] found that using a high concentration of SDF caused solid fluoride to precipitate inside the SDF treated samples and that there was a significant link between the percentage of crystal size and the quantity of SDF. SDF also releases fluoride ions and aids in the deposition of silver phosphate to restore mineral content, leading in the re-hardening of soft tooth structure [17].

Also, the results of this study revealed that the treatment of the carious dentine with SDF resulted in significant increase of dentine remineralization with increase in Ca/P ratio from 1.47±0.04 to 1.92±0.35. This results in agreement with the results of Mei et al. [12], who studied extracted primary teeth with cavities of children who received biannual applications of SDF using SEM, and they found the arrested lesions were remineralized by calcium and phosphate ions and had intact collagen fibers.

The previous studies suggested that the SDF provides alkaline environment that promotes the formation of covalent bond between phosphate ions from saliva and the intact collagen which becomes binding site for calcium ions which leads to apatite nucleation through the collagen [12,19]. Also, the previous in vitro studies were suggested that demonstrated that SDF, reduces dentin demineralization, enhances tooth remineralization, increases the pH of carious biofilm [12,13].

One of the key explanations for the stoppage of caries lesions treated with SDF might be the preferential precipitation of fluorohydroxyapatite with lower solubility. This was corroborated by Mei et al. [18], who found that the chemical environment of the phosphate functionality altered in all SDF groups, and SDF interacted with calcium and phosphate ions to create fluorohydroxyapatite.

CONCLUSION

Within the limitation of this in vitro study the following conclusion can be drawn, Silver diamine fluoride (SDF) was able to remineralize carious primary dentine.

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