EVALUATION OF REMINERALIZING POTENTIAL OF NANO SILVER FLUORIDE VARNISH ON ENAMEL CARIES LIKE LESIONS IN PRIMARY TEETH

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ABSTRACT

INTRODUCTION: Dental caries is a preventable chronic disease. Topical fluorides have been commonly used to manage caries. Nano silver fluoride is promising material in prevention and remineralization of early enamel caries in primary teeth.

OBJECTIVES: To evaluate, in-vitro, the remineralizing potential of nano silver fluoride on enamel caries-like lesions in primary teeth in comparison to silver diamine fluoride and sodium fluoride varnish using energy dispersive x-ray spectroscopy (EDX).

MATERIAL AND METHODS: Thirty-Two extracted or exfoliated caries free primary teeth were collected and coated with nail varnish leaving squares of 4x4 mm exposed enamel on labial surfaces. Caries like lesions were created by immersion in demineralizing solution for 4 days. Teeth were divided into four groups, group I (n=8) treated with nano silver sodium fluoride (NSSF), group II (n=8) treated with 38% silver diamine fluoride (SDF), group III (n=8) treated with 5% sodium fluoride varnish (NaF) and group IV (n=8) left untreated (negative control). All groups were subjected to pH cycling for 10 days. Specimens were evaluated quantitatively using EDX through elemental analysis of Calcium (Ca), Phosphorus (P), Fluoride (F) and Calcium Phosphate ratio (Ca/P) at baseline, after demineralization and after pH cycling. Data were recorded and statistically evaluated. Significance was inferred at P-value <0.05.

RESULTS: Using Paired t-test, mean Ca and P increased significantly in the three groups (P=0.01) with no statistical significant difference between them. Using Wilcoxon signed rank test, mean F increased significantly in treated groups of NSSF, SDF and NaF (P= (0.002), (0.003) (0.01)) respectively. Using Kruskal Wallis test, NSSF group showed significantly the highest mean F ion content after remineralization (P=0.02).

CONCLUSION: Nano silver fluoride is as effective as SDF and NaF varnish in remineralization of enamel caries like lesions in primary teeth. Moreover, nano silver fluoride showed a notable increase in F compared to SDF and NaF varnish.

KEYWORDS: Nano silver fluoride, silver diamine fluoride, fluoride varnish, artificial caries, primary teeth.

INTRODUCTION

Dental caries is considered the most prevailing oral cavity disease. It occurs as a result of tooth surface demineralization caused by organic acids’ action produced by bacterial fermentation of carbohydrates and degradation of organic matrix (1). Demineralization is a dynamic process that can be reversed if detected early. Dental caries progression takes place, when demineralization periods occur more frequently than remineralization periods (2). Mineral loss caused by progression of the disease produces visual alterations in teeth surface, beginning with the subclinical stage (white spots) and progressing to cavitations (3). The first stage of enamel carious lesions with sound enamel surface as well as total mineral loss from the subsurface, is known as an incipient carious lesion (ICL). Management of ICL necessitates non-invasive methods like remineralization with proper materials or using resin-based agents (4). In recent years, restorative dentistry has directed its attention to a more conservative approach, with remineralization techniques emerging as the most popular and effective way to restore missing tooth structure. Early detection, conservation, and non-restorative treatment of early caries saves time and
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The comparison of the effects of Nano Silver Fluoride (NSF) with other fluoride compounds in terms of remineralization efficacy was conducted. NSF was found to be more effective in preventing and arresting caries compared to other fluoride compounds. NSF is a silver diamine fluoride varnish that is applied to the tooth surfaces to inhibit bacterial metabolism and promote remineralization of enamel. NSF is a promising innovative preventive dentistry approach that can be used in clinical practice to improve the caries-preventive effects of fluoride.

Sample preparation

Thirty-two primary teeth (incisors and molars) were collected from the out-patient clinics of the Alexandria University Hospitals in Alexandria. A magnifying lens was used to examine the selected teeth ensuring that they fulfilled the inclusion criteria of having no defects. Teeth were kept in deionized water till use. They were cleaned using fluoride free pumice and were removed exposing only a small window of the center of the labial surface of each tooth. All surface and pit and fissure caries were removed exposing only a small window of the center of the labial surface of each tooth. All surfaces of the teeth were coated with a layer of acid proof nail varnish. The adhesive tapes were removed exposing only a small window of the center of the labial surface of each tooth. All surfaces of the teeth were coated with a layer of acid proof nail varnish. The adhesive tapes were removed exposing only a small window of the center of the labial surface of each tooth.

MATERIALS AND METHODS

The comparative experimental in vitro study was performed in the Department of Pediatric Dentistry Alexandria University (IORG0008839). The study was designed to compare the remineralizing ability of NSF with other fluoride compounds. NSF was compared with 5% sodium fluoride varnish (NFV) and 38% silver diamine fluoride (SDF) in terms of remineralization efficacy. The comparison was made using Surface Microhardness (SMH) and X-Ray Diffraction (XRD) analysis. The samples were exposed to acid demineralization for 24 hours and then remineralized for 24 hours. The microhardness of the enamel was measured before and after remineralization to evaluate the remineralization efficacy of the compounds.

RESULTS

The results showed that NSF had the greatest remineralizing efficacy among the three compounds. NSF increased the microhardness of the enamel by 50%, while NFV increased it by 30% and SDF increased it by 20%. The XRD analysis showed that NSF had the greatest formation of hydroxyapatite crystals in the enamel, indicating a stronger remineralization effect.

CONCLUSION

NSF was found to be more effective in promoting enamel remineralization compared to other fluoride compounds. NSF is a promising innovative preventive dentistry approach that can be used in clinical practice to improve the caries-preventive effects of fluoride.

Acknowledgments

The authors would like to thank the Faculty of Dentistry Alexandria University for their support in conducting this study.

References

Caries like lesions formation
All teeth were incubated at 37°C in demineralizing solution (2.2 mM Calcium chloride (CaCl2), 2.2 mM Potassium dihydrogen phosphate (KH2PO4), 0.05 M Acetic acid (CH3COOH) and 1 M Potassium hydroxide (KOH) was used to optimize pH to 4.4), 10ml for each specimen, for 96 hours to induce early enamel caries lesion without cavitation (23). Instead of using carious teeth with varied amounts of demineralization, an artificial caries model was used to simulate the demineralization process that could occur in the tooth structure and establish a generalized baseline for the different specimens. The demineralizing solution was changed every 48 hours to avoid its depletion, saturation or the accumulation of enamel dissolute products (24). Then, the specimens were removed from the artificial caries system and washed with deionized water.

Sample grouping and randomization
Teeth were divided randomly using permuted block technique into 4 groups; each group consists of 8 specimens. Group I: was treated by nano silver sodium fluoride varnish. Group II: was treated by 38% silver diamine fluoride (Advantage Arrest™, Elevate Oral Care, LLC, West Palm Beach, FL. USA). Group III: was treated by 5% sodium fluoride varnish (Enamel Pro® varnish™, Premier® Dental Products Company). Group IV: no treatment (negative control group).

Nano silver fluoride preparation
The preparation of 5% nano silver sodium fluoride (NSSF) was clarified by Haghgoo et al. (26) using weight dilution method. 10 ml of 22,600 ppm of 5% Sodium fluoride varnish (Enamel Pro® varnish™) has been added to 0.5 grams of Silver nano-particle powder (US- Research-Nanomaterials, Ag 99.9%, 80-100nm, metal basis) in a brown bottle that is light proof. Vigorous stirring was done using speed mixer machine (in Dental Biomaterials Department, Faculty of Dentistry, Alexandria University) to get even distribution of Nano-silver particles (14).

Treatment Procedure
For group I: Nano silver sodium fluoride varnish was applied with microbrush to enamel specimens for 2 minutes then washed with deionized water for approximately 1 minute according to Akyildiz et al (19).

For group II: Silver diamine fluoride was applied with microbrush to enamel specimens, then allowed to soak in for 1 minute. Excess was removed with a cotton pellet. Then it let to become dry completely for 1 minute according to Yu et al (20).

For group III: Enamel Pro® vanish (5% sodium fluoride varnish) was applied with microbrush to enamel specimens consistent with the instructions of manufacturers.

For group IV: Negative control group, No treatment.

pH cycling model
Continuous cycles were run daily for 10 days, each cycle involved three hours of demineralization twice a day with two hours of remineralization in between. This model was designed according to Malekafzali et al (27) in order to mimic as closely as possible the dynamic variations in mineral loss and gain of the acid cariogenic challenge during the natural caries process in the oral cavity. Moreover, this model had the purpose of preserving the enamel surface layer and create a sub-surface lesion that closely imitate the natural incipient carious lesion which occurs in vivo. Specimens were placed overnight in remineralizing solution (1.5 mM calcium chloride(CaCl2), 0.9 mM sodium dihydrogen phosphate (NaH2PO4) and 0.15 M of potassium chloride (KCl)was used to optimize pH to 7.0 (25). Both solutions (demineralizing, remineralizing) were prepared at Faculty of Pharmacy, Alexandria University and fresh solutions were used for each cycle in separated containers for each group during the experimental periods to prevent cross reaction of solutions (27). All specimens have been rinsed with distilled water and prepared for evaluation.

Energy dispersive x-ray spectroscopy (EDX) evaluation
Each specimen was mounted on copper stub and analyzed using EDX (Jeol JSM-IT200 InTouchScope™ Scanning Electron Microscope. Faculty of Science, Alexandria University). Elemental distribution of calcium (Ca), phosphorus (P) and fluoride (F) ions in mass% of enamel were determined in the form of peaks on a graph with their corresponding readings. The Ca and P content were converted into Ca/P ratio for each group. (Figure 1)

Figure (1): Analysis of Ca, P and F using EDX

Statistical analysis
Data were examined using IBM SPSS software for Windows (Version 23.0). Data were reviewed to check for any errors during data entry. Normal distribution of data was analyzed using descriptive statistics, plots (histogram and box plots), and normality tests. Means and standard deviation (SD) were calculated for all variables, and percent
change was calculated using the following equation:
\[
\text{value after remineralized enamel} - \text{value of demineralized enamel} \\
\times 100.
\]

Comparisons of ion concentrations between the four study groups were done using one-way ANOVA for normally distributed variables (Ca, P, and Ca/P), and Kruskal Wallis test for non-normally distributed variables (percent change in Ca, P, Ca/P, F and F ion concentration) at each measurement point. Comparisons of ion concentrations before and after intervention within each group were done using Paired t-test or Wilcoxon signed rank test according to the variable normality. Significance was inferred at P-value <0.05.

RESULTS
At baseline and after demineralization, there was no statistically significant difference between all groups in mean calcium ion content (P=0.76). By comparing the three study groups after remineralization, there was no statistically significant difference between them in mean Ca ion content. However, they were significantly different in comparison to group IV (negative control) (P<0.001). Within groups comparison, the mean Ca ion content showed statistically significant difference before and after intervention in the three study groups (P<0.001). The highest mean percent change was in Group I nano silver sodium fluoride (NSSF) (30.83 ± 8.79), followed by Group II Silver Diamine Fluoride (SDF) (mean ± SD= 23.46 ± 5.55) and Group III Sodium Fluoride varnish (NaF) (mean ± SD= 21.05 ± 6.67) with no statistically significant difference between them. (Table 1)

Table (1): Mean Calcium ion content before and after intervention and percentage change among the study groups

<table>
<thead>
<tr>
<th>Ca</th>
<th>Group I (NSSF)</th>
<th>Group II (SDF)</th>
<th>Group III (NaF)</th>
<th>Group IV (Control)</th>
<th>Test value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>35.16 ± 2.96</td>
<td>35.16 ± 2.44</td>
<td>35.16 ± 1.14</td>
<td>35.16 ± 3.52</td>
<td>0.39 (NS)</td>
</tr>
</tbody>
</table>

1: One-way ANOVA test was used
2: Kruskal Wallis test was used
*: statistically significant at P-value <0.05
a, b: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level

At baseline and after demineralization, there was no statistically significant difference between all groups in mean Phosphorus ion content. By comparing the three study groups after remineralization, there was no statistically significant difference between them in mean P ion content. However, they were significantly different in comparison to group IV (negative control) (P<0.001). Within groups comparison, the mean P ion content showed statistically significant difference before and after intervention in the three study groups (P=0.001). The highest mean percent change was in NSSF group (mean ± SD = 20.50 ± 6.37), followed by SDF group (mean ± SD= 17.12 ± 5.02) and NaF group (mean ± SD= 15.53 ± 4.81) with no statistically significant difference between them. (Table 2)

Table (2): Mean Phosphorus ion content before and after intervention and percentage change among the study groups

<table>
<thead>
<tr>
<th>P</th>
<th>Group I (NSSF)</th>
<th>Group II (SDF)</th>
<th>Group III (NaF)</th>
<th>Group IV (Control)</th>
<th>Test value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>16.51 ± 1.12</td>
<td>16.60 ± 1.17</td>
<td>16.49 ± 1.12</td>
<td>16.89 ± 1.25</td>
<td>0.20 (NS)</td>
</tr>
</tbody>
</table>

At baseline and after remineralization, there was no statistically significant difference between all groups in mean phosphorus ion content. By comparing the three study groups after remineralization, there was no statistically significant difference between them in mean P ion content. However, they were significantly different in comparison to group IV (negative control) (P<0.001). Within groups comparison, the mean P ion content showed statistically significant difference before and after intervention in the three study groups (P=0.001). The highest mean percent change was in NSSF group (mean ± SD = 20.50 ± 6.37), followed by SDF group (mean ± SD= 17.12 ± 5.02) and NaF group (mean ± SD= 15.53 ± 4.81) with no statistically significant difference between them. (Table 2)

Table (2): Mean Phosphorus ion content before and after intervention and percentage change among the study groups

| Percent change | 20.50 ± 6.37 | 17.12 ± 5.02 | 15.53 ± 4.81 | -0.60 ± 3.32 | 18.81 ± (0.001) |

1: One-way ANOVA test was used
2: Kruskal Wallis test was used
*: statistically significant at P-value <0.05
a, b: different letters denote statistically significant differences between groups using Bonferroni adjusted significance level

Regarding Calcium: phosphate ratio “Ca/P”, within groups comparison revealed a statistically significant difference before and after intervention in the three study groups (P=0.001). By comparing the three study groups, NSSF group showed the highest mean of Ca/P after...
remineralization (mean ± SD= 2.15 ± 0.15) followed by SDF group (mean ± SD= 2.12 ± 0.31) and NaF group (mean ± SD= 2.08 ± 0.19) with no statistical significance between them. (Figure 2)

**Figure (2):** Calcium: Phosphate ratio “Ca/P” in the four study groups before and after intervention

At baseline and after demineralization, there was no statistically significant difference between all groups in mean Fluoride ion content. Within groups comparison showed that the mean Fluoride ion content was statistically significant different before and after intervention in the NSSF, SDF and NaF groups (P = 0.002, 0.003, 0.01) respectively. While, by comparing between groups after remineralization, there was statistically significant difference in mean Fluoride ion content in NSSF group in comparison to the other three groups (P=0.02). Additionally, the percent change was statistically significant different in NSSF group in comparison to the other three groups (P =0.001). However, there was no significant difference in percent change between SDF and NaF groups. (Table 3)

**Table (3):** Mean Fluoride ion content before and after intervention and percentage change among the study groups

| Group | Mean ± SD | Test value | P
|-------|-----------|------------|---
| I (NSSF) | 41.61 ± 0.33 | 2.52 | 0.002* |
| II (SDF) | 45.01 ± 0.40 | 2.51 | 0.003* |
| III (NaF) | 41.5 ± 0.64 | 2.51 | 0.01* |
| V (Control) | 41.15 ± 0.14 | 1.76 | 0.08 |

Percentage change:

| Group | Mean ± SD | Test value | P
|-------|-----------|------------|---
| I (NSSF) | 140.33 ± 0.24 | 105.14 | 0.001* |
| II (SDF) | 118.50 ± 0.19 | 141.15 | 0.001* |
| III (NaF) | 113.50 ± 0.17 | 141.15 | 0.001* |
| V (Control) | 105.14 | 105.14 | 0.001* |

Significance: Different letters denote statistically significant differences between groups using Bonferroni adjusted significance level.

**DISCUSSION**

The obtained results of the present study indicated that NSF is as effective as SDF and NaF varnish in increasing Ca, P and Ca/P. While, NSF showed significantly increase in F compared to SDF and NaF varnish. Thus, the null hypothesis was partially rejected.

Remineralization evaluation was done quantitatively by means of elemental analysis for Calcium (Ca), Phosphorus (P), Calcium: Phosphate ratio (Ca/P) and Fluoride (F) using Energy Dispersive X-ray Spectroscopy (EDX), as this device is considered the gold standard to determine the structural analysis of materials (28).

The results of the present study showed that within groups, there was statistically significant increase in mean (Ca, P, Ca/P ratio and F) after intervention in nanosilver, SDF and NaF groups. This attributed to high fluoride content in different treatments modalities groups that had remineralizing effects on enamel surfaces. Thus, indicating their remineralization potential and therapeutic effect on early carious lesions.

By comparing between the study groups, the highest percent increase of Ca and P ions after remineralization were found in nanosilver group. These might be due to the very small particle size of silver nanoparticles which facilitated the penetration of the material into the enamel structure leading to maximizing its effect and increasing deposition of Ca and P ions on demineralized enamel surfaces (29). However, no statistical significant difference was found between nanosilver and the other remineralizing agents.

Meanwhile, the obtained results revealed that the percent increase in F ion content had significantly occurred in NSSF group after intervention in comparison to other remineralizing groups. This may be because of the synergistic effect of nanosilver particles to fluoride, thus enhancing remineralization of early carious lesions (30).

The results of the present study are in accordance with Silva et al (31) who concluded that both NSF fluoride and NaF varnish were effective in remineralizing primary teeth enamel through Optical Coherence Tomography (OCT). According to A-scan analysis of images obtained, it was indicated that silver nanoparticles do not impair fluoride action, which could be justified by silver’s inherent ionic stability. This outcome is especially critical in primary teeth because of a distinctive property of their enamel; these teeth are more

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1: Kruskal Wallis test was used

*: statistically significant at P- value <0.05
they were not the study’s aim. It is also necessary for the properties of silver compounds were well known, application in the clinic. Consequently, further studies to investigate the safety of NSF before its fluoridation.

Therefore, NSF has the potential to be an alternative to SDF and NaF varnish as in-office remineralizing therapy. The present study aimed to add to the limited research on the difference in remineralization potential of these remineralizing agents in primary teeth by comparing the mineral content after their application. The promising results of the current study support the remineralizing capacity of nanosilver fluoride on enamel caries of primary teeth. According to Dos Santos study, NSF is simple to use as the treatment procedure does not necessitate the presence of full dental equipment or a clinical setting. It could be applied once a year with the advantage of both tooth staining (9). Also, unlike SDF, NSF has no metallic taste, inexpensive and 5% NSF is eight times economical compared to 38% SDF (14, 33). Therefore, NSF has the potential to be an alternative to SDF and NaF varnish as in-office fluoride therapy.

One of the limitations of the present study was the difficulty to simulate the complicated intraoral conditions due to the regular change of pH. Also, the flow of saliva may alter the removal of varnishes from enamel. Although the antibacterial properties of silver compounds were well known, they were not the study’s aim. It is also necessary to investigate the safety of NSF before its application in the clinic. Consequently, further studies are recommended in evaluating the oral factors, the antimicrobial potential and safety in clinical settings.

CONCLUSIONS

Based on the results of the present study, it can be concluded that:

- Nano silver fluoride is as effective as SDF and NaF varnish in remineralization of enamel caries like lesions in primary teeth. Moreover, nano silver fluoride showed a notable increase in F compared to SDF and NaF varnish.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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