ORIGINAL PAPER

COMPARISON OF NANO-SILVER FLUORIDE, NANO-HYDROXYAPATITE AND SODIUM FLUORIDE VARNISHES IN PREVENTION AND ARRESTMENT OF INITIAL CARIOUS LESIONS: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introduction: Globally, the increased prevalence of dental caries in children makes the need for dental re-mineralizing agents necessary. To the best of our knowledge there are a few published clinical trials on the effectiveness of nano-re-mineralizing agents but without comparing between them.

Objectives: To compare nano-silver fluoride (NSF), nano-hydroxyapatite (n-HAP) and sodium fluoride (NaF) varnishes in prevention and arrestment of initial enamel caries on young permanent teeth.

Material and methods: One hundred and fifteen children with ages ranging from 7 to 10 years were randomly divided into three groups: the NSF group (n = 39), the n-HAP group (n = 37) and the NaF group (n = 39). The clinical evaluation of dental surfaces was done by one masked examiner using ICDAS II to detect the number of new and arrested initial lesions. Varnish application was done annually for NSF, but it was done biannually for n-HAP and NaF. This was followed up at 3, 6 and 12-month intervals. The Kruskal-Wallis test was used to compare the groups.

Results: After a follow-up period of 12 months, the NSF group had a significantly lower median number of new carious lesions, 1 (0), than the n-HAP group, 2 (3), with p = 0.01. There were significantly more arrested lesions in both NSF and NaF than in n-HAP at all visits, with p = 0.003, 0.01 and 0.002. At the level of children, the NSF group had the lowest number of children who were affected by new caries (23.1%) and the highest number of children who have arrested initial caries (87.2%).

Conclusions: Annual application of NSF varnish might be recommended as an effective method of dental caries prevention and treatment of initial caries lesions.

Key words: clinical trial; children; initial caries lesion; arrestment; nano-silver fluoride; fluoride varnish.

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INTRODUCTION

Dental caries is a chronic, multifactorial, transmissible and infectious disease which occurs as a result of a shift in the demineralization/re-mineralization equilibrium favoring net demineralization of the enamel. It is still a major health problem that affects primary and permanent teeth in deprived parts of the world despite dental care improvements [1]. Approximately, 2.43 billion people (36% of the population) in the world have dental caries in their permanent teeth [2].

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The initial carious lesion can be defined as a primary lesion which has not reached the stage of an established lesion with cavitation. Prevention and arrestment of initial carious lesions by re-mineralizing agents is required in order to reduce the need for therapeutic intervention, which is costly and difficult in many cases among children [3]. The application of topical fluoride can prevent dental caries by inhibiting demineralization of the crystal structures inside the tooth and enhancing remineralization [4]. Sodium fluoride varnish (NaF) is used as a type of topical fluoride treatments, and it is a safe way to protect the teeth whilst providing the highest and safest possible fluoride concentration. Furthermore, its sticky nature bonds with the tooth surface for several hours. Hence, this will prevent new carious lesions and extension of previous caries [5]. However, the fluoride depends on the quantity of calcium and phosphate ions obtainable in the oral fluid, thereby limiting remineralization [6].

The synthetic hydroxyapatite is a biocompatible and bioactive material and it is widely used in medicine and dentistry. This compound has a similar chemical composition to enamel but with larger particle size. Therefore, it is not suggested to repair the damaged enamel directly [7]. With the emergence of nanotechnology in the 21st century, nano-hydroxyapatite (n-HAP) has been synthesized to possess similar properties as a biological apatite, and it also has a higher solubility, surface energy and bioactivity in order to re-mineralize initial enamel caries [8]. However, its application in preventive dentistry should be investigated further.

A silver based preparation, nano-silver fluoride (NSF) was introduced by Santos *et al.* [9] to combat the adverse effects of silver diamine fluoride (SDF) such as black discoloration of carious tissue and ulceration [10]. NSF is a new experimental formulation containing silver nanoparticles, chitosan and fluoride that have preventive and antimicrobial properties. It is safe to be used in humans and has effective antimicrobial properties against cariogenic bacteria [11]. NSF is available as a reddish yellow solution that proves to be stable for three years, low in cost and it does not stain the teeth [12, 13].

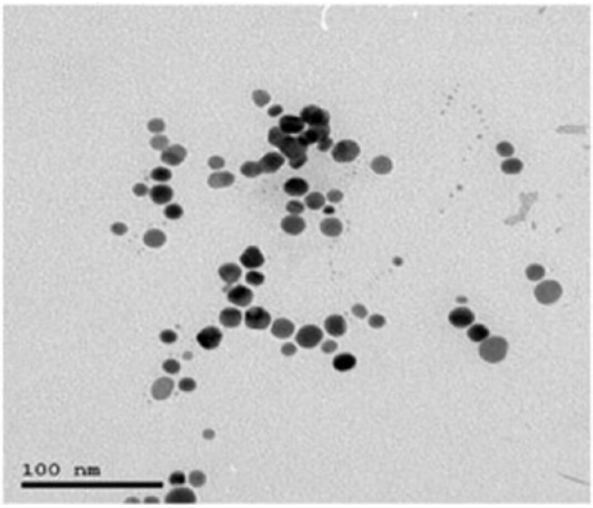

FIGURE 1. Transmission electron microscopy (TEM) images of the prepared nano-silver fluoride (NSF)

To the best of our knowledge, most studies that have evaluated the effectiveness of nano-anticaries agents (NSF and n-HAP) were in-vitro studies. In addition, there have been few published clinical trials on the effectiveness of these agents and no comparisons were made.

OBJECTIVES

The aim of the present study was to compare between NSF, n-HAP and NaF varnishes in arrestment (inactivation) of initial enamel caries and preventing new caries in young permanent teeth through checking the value of arrested initial caries and new caries at the level of dental surfaces and at the level of children.

MATERIAL AND METHODS

RE-MINERALIZING AGENTS

One of the three dental varnishes compared in this study was commercially available and the others were made specially for this study.

1) Preparation of NSF varnish: Targino *et al.*'s [13] formulation was followed by the Nano Gate Company in Cairo, Egypt (https://nanogate-eg.com/en/) in the preparation of NSF varnish. The chemical reduction of silver nitrate ($AgNO_3$) with sodium borohydride ($NaBH_4$), and the stabilizing substance chitosan biopolymer was used to create silver nanoparticles. Melting chitosan (2.5 mg/ml) in a 1% acetic acid solution was done. $AgNO_3$ (1 ml, 0.11 M) was added to the solution, and the mixture was stirred magnetically until homogeneous. After that, the liquid was poured into an ice-cold bath, and freshly made $NaBH_4$ (0.3 ml, 0.8 M) was added while vigorously swirling. The shift in color of the solution from colorless to light yellow and finally reddish signaled the beginning of the reduction of Ag^+. After that, the flask was taken out of the ice bath, and sodium fluoride varnish (containing 22,600 ppm of fluorine) was added to increase the solution's stability. Silver nanoparticles 400 µg/ml, sodium fluoride 22,600 ppm (µg/ml), and chitosan 2334 µg/ml are all present in the produced solution.

Transmission electron microscopy (TEM) was used to measure the size and form of silver nanoparticles. The analysis showed that 99% of the silver nanoparticles were spherical and had a particle size of 10 ± 2.0 nm as in Figure 1.

2) Preparation of n-HAP varnish: Nano gate Company in Cairo, Egypt (https://nanogate-eg.com/en/) produced

hydroxyapatite nanoparticles using the wet chemical approach, largely following Ferraz *et al.* [14]. At room temperature, calcium chloride was vigorously agitated in an aqueous solution. The calcium chloride solution was first progressively diluted with a solution of ammonium hydrogen phosphate, and then the pH was adjusted to 10 by dilution with an ammonia solution. After reaction at 40°C for 4 hours, the reaction continued and was stirred for an additional 16 hours at room temperature. The n-HAP white precipitate developed.

$10Ca(OH)_2 + 6H_3PO_4 \rightarrow Ca_{10}(PO_4)6(OH)_2 + 18H_2O$

To formulate 60 ml of n-HAP 10% varnish, 6 g of n-HAP powder was weighed out on a sensitive balance (WTC 200, RADWAG, Poland) and dispersed in 60 ml of deionized water with stirring using a hot plate and stirrer (MSH-20A, Wetige®, Germany) for 30 min then 2.4 gm of carboxymethyl cellulose (Loba Chemie, India) was scattered gradually and gently over the solution under mild temperature with strong stirring to get a homogeneous varnish.

TEM was used to assess the HAP's n-size and form. According to the analysis, n-HAP was white needle-like and 150 nm ± 30 nm in size, as in Figure 2.

3) NaF varnish (Polimo dental varnish, Imicryl, Turkey): Commercially available in a disposable package of 0.4 ml, this varnish contains 5% sodium fluoride. It was chosen in accordance with a recently published study that examined the antibacterial effects of three types of common fluoride varnishes (Polimo dental varnish, Fluoro Dose varnishes, and MI varnish containing calcium phosphopeptide-amorphous calcium phosphate (CPP-AC) by measuring the diameter of the inhibition zone against two cariogenic bacteria, *Streptococcus mutans* (S.M.) and *Lactobacillus acidophilus* (L.A.), and it was concluded that the use of these varnishes seems to be appropriate for preventing dental caries [15].

CLINICAL TRIAL

This study was a prospective, controlled, and randomized clinical experiment. The ethical committee of the Faculty of Dentistry, Mansoura University granted consent with approval number A01071221 and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki, 2013). The trial took a place in pediatric dental clinics of the Faculty of Dentistry in the period from January 2021 to March 2022. The parents of the participating children were asked to sign informed consent, after being informed of the study objectives, risks and benefits.

PARTICIPANTS SELECTION

The participants of this study were 115 children aged 7-10 years, selected from the children who attended for dental treatment in the pediatric dentistry clinic.

FIGURE 2. Transmission electron microscopy (TEM) images of the prepared nano-hydroxy apatite (n-HAP)

Inclusion criteria were as follows: healthy children aged 7 to10 years with moderate or high risk of developing caries, as determined by the American Dental Association's caries risk assessment form, and having at least one permanent tooth with a score of 0 and score of 1 or 2 according to visual scoring criteria of International Caries Detection and Assessment System II (ICDAS II) [16].

Children were excluded if they had used a re-mineralizing agent other than regular toothpaste in the previous three months, with allergy to silver particles or with any intraoral pathology.

CALCULATING THE SAMPLE SIZE

Based on previous studies [9, 17], the sample size was calculated using the G*Power program version (3.1.9.7; Heinrich-Heine-University Dusseldorf, Dusseldorf, Germany). It was estimated that 36 children in each group were sufficient to generate statistically significant data with power of 95% and α error = 0.05. In addition, to make up for possible losses (drop out), it was decided to include 40 participants in each group.

RANDOMIZATION

Initially 150 children were screened. 120 of them matched the inclusion criteria and were included. Block randomization was created by using a computerized randomization web site (https://www.sealedenvelope.com/simple-randomiser/v1/lists) to ensure that the number of participants in each group was distributed similarly. The generated list of participants' numbers with their codes A, B and C was kept with the dentist who was responsible for varnish application. Each child was given his number without the code written on his record-

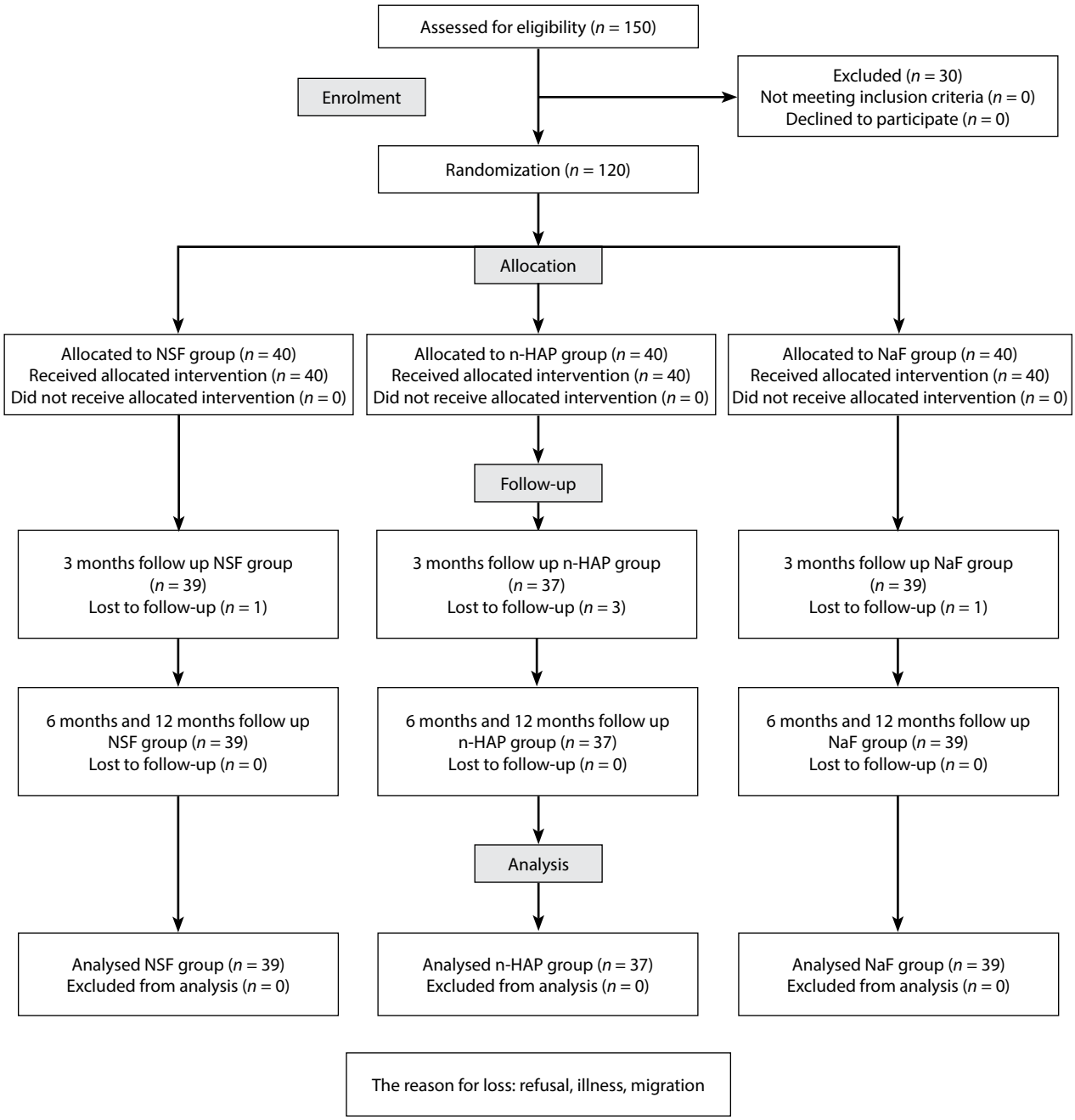

FIGURE 3. Evaluation, enrolment, randomization, dropout, and completion of participants

ing sheet. Codes A, B and C referred to NSF, n-HAP and NaF, respectively. The actual number of children who attended all follow-up visits was 115, as shown in the CONSORT flowchart in Figure 3.

CONDUCTION OF THE STUDY

Dental examination: In the pediatric dentistry clinic, the dental examination was performed by one qualified and calibrated examiner in a dental chair while the subject was semi-supine, under good lighting, and while taking into account all techniques of infection control. Clinical examination was carried out using a dental mirror, ball ended pig tailed explorer, gloves, mask, and dental examination sheets. The study included the first visit and three follow-up visits; three months, six months, and twelve months following the baseline visit. Evaluations at all visits were carried out by the same examiner, who was unaware of the treatment assignment and had no access to any of the outcomes from earlier visits. The intra-examiner reliability of score records was con-

firmed and tested by Cohen's kappa test, and the intra-examiner agreement was determined to be 80%. 10% of the sample was randomly chosen to be re-examined for intra-examiner reproducibility.

A. Baseline evaluation

Clinical assessment was carried out using ICDAS II visual scoring criteria for determining the sound enamel and active initial caries lesions [16]. Scoring criteria are as follows:

- **score 0** represents sound tooth surface: no evidence of caries after 5 seconds of air drying;
- **score 1** represents first visual alteration in enamel: opacity or discoloration (white or brown) can be detected at the entrance to the pit or fissure seen after prolonged air drying;
- **score 2** represents distinct visual change in enamel: discoloration is apparent when it is moist, and lesion must be visible when dry.

Dental caries assessment was done for any surface of fully erupted permanent teeth which could be seen clearly and with a score of 0, 1 or 2. The type of recorded surfaces, at baseline, was written in a table related to each child in order to be a guide for follow-up assessment.

For each child the assessment was recorded as follows: the total number of surfaces with a score of 0 and the total number of surfaces with a score of 1 or 2 were considered as initial caries lesions; for example, a child has 5 surfaces of grade 0 and 4 surfaces of grade (1+2), and then the median of 0 and median of (1+2) were calculated for all children in the same group.

Method of varnish application. First, full mouth polishing with a rubber cup in a slow speed hand piece was carried out to remove any food debris or plaque present on the tooth surface, while applying a constant stream [18].

- The application of varnish was done for all dental surfaces.
- Isolation of the teeth was done with the help of cotton rolls and a saliva ejector.
- Using a disposable micro-applicator brush, approximately 0.4 mL of either NSF, n-HAP, or NaF varnish was applied quadrant-wise sequentially starting from the lower arch to all of the teeth. Whereas n-HAP and NaF varnishes were applied twice at baseline and at the 6-month follow-up [19], NSF varnish was only used once [10]. Furthermore, the children and their parents were instructed:

1. Not to rinse, drink or eat for at least 45 min.
2. Take liquid and semisolid diet for that day.
3. Not to brush the teeth for that day.

All children received a toothbrush, toothpaste with 1000 ppm fluoride, instructions on good dental hygiene, and advice on eating a balanced diet. The participants who required further filling or extraction treatment were treated in pediatric dental clinics in the Faculty of Dentistry, Mansoura University.

B. Follow-up evaluation

All children underwent clinical evaluations utilizing visual and tactile inspection after 3, 6 and 12 months for detecting and recording the number of new lesions and inactive initial lesions. The characteristics of active and inactive lesions were determined according to the International Caries Classification and Management System (ICCMS) [20], as follows:

- Active lesion: As the tip of the ball-ended probe is gently rubbed across the enamel's surface, it feels rough due to the enamel's whitish/yellowish surface being opaque and losing its brilliance.
- Inactive lesion: When the ball-ended probe's tip is gently dragged across the enamel's surface, it feels smooth, shiny, firm, and white.

The children next received varnish applications at the 6-month visit. Regarding the NSF group, water was applied, so that the participants and their parents were under the impression that there was no difference between the three groups.

This clinical trial followed the CONSORT recommendations and was registered at www.clinicaltrials.gov with the protocol NCT04887389.

Blinding. The children and their parents were blinded to the specific names of dental varnishes, whilst the group assignment was hidden from the calibrated examiner. Since the color of the three agents was different, blinding related to the investigator who applied the varnish was impossible.

STATISTICAL ANALYSIS

With the help of IBM SPSS for Windows (Armonk, NY: IBM Corp. released 2019 version 26.0.), data were gathered, coded, input, and analyzed. The Kolmogorov-Smirnov test was used to test the distribution of quantitative data, and the median and inter-quartile range (IQR) were used to summarize non-normally distributed data. The Kruskal-Wallis test was used to compare the data in three groups, and pairwise comparisons were performed for significant outcomes. The χ^2 test was used to compare the qualitative data between the three groups. Qualitative variables were reported as numbers and percentages. Cohen's κ statistic was applied to assess the intra-examiner reliability. The allowed margin of error was 5%, and the significance level for the p-value was set at $p > 0.05$. The baseline characteristics (age, sex, and recording scores) of studied groups were compared by statistical tests prior to follow-up visits in order to ensure that no significant difference was found among the three groups. The data were analyzed at the level of teeth surfaces and at the level of children.

RESULTS

The present study was a randomized clinical trial. The study included a total of 115 patients aged 7-10

years old divided into three studied groups according to the type of dental varnish used as follows: NSF group – 39 children with mean age 8.8 ± 1.3, 23 males and 16 females; n-HAP group – 37 children with mean age 8.4 ± 1.2, 13 males and 24 females; NaF group – 39 children with mean age 8.3 ± 1.2, 19 males and 20 females. The results showed no statistically significant difference in the mean age and percentage of gender or in the clinical ICDAS scores (0, 1 and 2) between the three groups as shown in Table 1.

NEW LESIONS AND ARRESTED (INACTIVE) INITIAL LESIONS AT LEVEL OF PERMANENT SURFACES

The median number and IQR of new carious lesions showed no statistically significant difference between the three groups in 3- and 6-month follow-up visits, while at the 12-month follow-up the NSF group had significantly fewer new carious lesions than in the n-HAP group ($p = 0.01$) as shown in Table 2.

By comparing the median and IQR of inactive initial lesions, the results showed statistically significantly more inactive lesions in both NSF and NaF than n-HAP in all follow-up visits with $p = 0.003$, 0.01 and 0.002 at 3-, 6- and 12-month visits respectively. There were significant differences between NSF and n-HAP groups ($p = 0.005$, 0.01, 0.009) and also between NaF and n-HAP groups ($p = 0.009$, 0.04, 0.004), while there was no statistically significant difference between NSF and NaF groups ($p = 1.0$) among all follow-up visits, as shown in Table 2.

NEW LESIONS AND ARRESTED (INACTIVE) INITIAL LESIONS AT LEVEL OF CHILDREN

The number of children with new caries lesions in the NSF group did not differ significantly from the number of children with new caries lesions in the n-HAP and in the NaF group at 3- and 6-month follow-up visits. At the 12-month follow-up the NSF group had the low-

TABLE 1. Baseline characteristics of studied groups.

Studied groups	NSF, $n = 39$	n-HAP, $n = 37$	NaF, $n = 39$	p-value
Age, mean ± SD	8.8 ± 1.3	8.4 ± 1.2	8.3 ± 1.2	0.223*
Gender, n (%)				
Male	23 (59)	13 (35.1)	19 (48.7)	0.114#
Female	16 (41)	24 (64.9)	20 (51.3)	
Number of permanent teeth surfaces with particular scores at baseline (ICDAS II)	$n = 39$ Median (IQR)	$n = 37$ Median (IQR)	$n = 39$ Median (IQR)	
Score 0	90.5 (7.0)	90.8 (7.8)	91.6 (8.3)	0.561Δ
Initial caries lesions (scores 1+2)	6.7 (5)	6.6 (6)	6.6 (7.6)	0.366Δ

**p-value of one-way ANOVA, #p-value of χ^2 test, Δp-value of Kruskal-Wallis test*
NSF – nano-silver fluoride, n-HAP – nano-hydroxy apatite, NaF – sodium fluoride varnish

TABLE 2. Comparing the number of new lesions and arrested initial lesions in the permanent surfaces among studied groups

Studied groups/Follow-up visits	NSF, $n = 39$, median (IQR)	n-HAP, $n = 37$, median (IQR)	NaF, $n = 39$, median (IQR)	p-value of Kruskal-Wallis test	P1, NSF vs. n-HAP	P2, n-HAP vs. NaF	P3, NSF vs. NaF
3 months							
New lesions	1 (1)	2 (1)	1 (1)	0.810	0.005*	0.009*	1.000
Arrested (inactive) initial lesions	3 (3)	2 (1)	3 (2)	0.003*			
6 months							
New lesions	1.5 (2)	2 (2)	1 (1)	0.109	0.012*	0.046*	1.000
Arrested (inactive) initial lesions	3 (3)	1.5 (1)	2 (3)	0.012*			
12 months							
New lesions	1 (0)	2 (3)	2 (1)	0.019 *	0.016*	1.000	0.089
Arrested (inactive) initial lesions	2 (3)	1 (1)	2 (2)	0.002 *	0.009*	0.004*	1.000

**p-value > 0.05 is considered statistically significant*
P1, P2 and P3 – pairwise comparison of Kruskal-Wallis test
NSF – nano-silver fluoride, n-HAP – nano-hydroxyapatite, NaF – sodium fluoride

TABLE 3. Comparing the number of children with new lesions and arrested initial lesions among studied groups

Studied groups/ Follow-up visits	NSF, *n* = 39 *n* (%)	n-HAP, *n* = 37 *n* (%)	NaF, *n*= 39 *n* (%)	*p*-value of χ^2 test	P1, NSF vs. n-HAP	P2, n-HAP vs. NaF	P3, NSF vs. NaF
Children with new lesions							
3 months	9 (23.1)	16 (43.2)	16 (41.0)	0.128			
6 months	10 (25.6)	12 (32.4)	12 (30.8)	0.794			
12 months	9 (23.1)	21 (56.8)	17 (43.5)	0.011*	0.003*	0.251	0.055*
Children with arrested initial lesions							
3 months	34 (87.2)	19 (51.4)	29 (74.4)	0.002*	0.001*	0.038*	0.151
6 months	34 (87.2)	16 (43.2)	29 (74.4)	< 0.001*	< 0.001*	0.006*	0.151
12 months	34 (87.2)	21 (56.8)	30 (76.9)	0.009*	0.003*	0.061	0.238

**p-value > 0.05 is considered statistically significant*
P1, P2 and P3 – pairwise comparison of χ^2 test
NSF – nano-silver fluoride, n-HAP – nano-hydroxyapatite, NaF – sodium fluoride,

est percentage of children with new caries lesions, significantly lower compared to n-HAP (23.1% vs. 56.8%, $p = 0.003$) and at the threshold of significance compared to the NaF group (23.1% vs. 43.5%, $p = 0.05$), as shown in Table 3.

With regards to the arrested initial lesions, the NSF group showed the highest number of children with inactive initial lesions (87.2%), significantly higher than the n-HAP group ($p = 0.001$, > 0.001, 0.003 for 3-, 6- and 12-month follow-up visits, respectively) and non-significantly higher than the NaF group ($p > 0.05$ for all follow-up visits), as shown in Table 3.

DISCUSSION

In this study, the three agents were in the form of varnishes, which allowed more prolonged exposure to the agent and less chair time required than that needed for foams and gels [21]. Young permanent teeth are at the highest risk of being affected by dental caries due to the more porous structure and higher protein content, so fluoride or calcium uptake could be increased when applied at that time [22].

The present study did not show statistically significant differences in the number of new and arrested incipient caries lesions between the NSF and NaF groups. However, the difference between the number of children with new carious lesions in both groups after 12 months was at the threshold of significance level (a trend towards superiority of NSF varnish over NaF varnish in the prevention of dental caries). Moreover, considering the annual application of NSF varnish versus the biannual application of NaF varnish, a single intervention with the NSF varnish seems more cost-effective.

The preventive and arrested effectiveness of NSF, as proved in this study, could be explained by the antimicrobial and re-mineralizing effect [12, 23]. The small size of the first component (silver nanoparticles) increases the surface area exposed to the microbe and forms free radicals, damaging the bacterial cell membrane and making it porous, leading to cell death.

Furthermore, silver ions could cooperate with sulfuryl groups during protein synthesis and inhibit the replication of DNA [12, 24]. The second component of NSF varnish is chitosan, which exhibits significant antibacterial and plaque reduction action. *In vitro* studies showed that chitosan interferes with the demineralization of the tooth enamel, inhibiting the release of mineral elements [25]. The third element is fluoride, which enhances NSF's action, inhibiting demineralization and increasing remineralization of tooth enamel and reducing the metabolism of the cariogenic bacteria. Fluoride has been demonstrated to inhibit the mechanism by which cariogenic bacteria metabolize carbohydrates to generate acids and thus affects adhesive polysaccharides bacterial growth [1, 26, 27].

Up to now, no published clinical trial has compared the efficacy of NSF and commercial fluoride varnish used on young permanent teeth over a 12-month period.

Results of the shorter (three months and three weeks) study by Girón *et al.* [28] suggest that adding nano-silver particles to a fluoride varnish significantly increases the effectiveness of the varnish in arresting white spot lesions in primary dentition.

Similarly, Waikhom *et al.* [24], who investigated the antimicrobial effectiveness of NSF, NaF, and chlorhexidine varnishes on the level of *Streptococcus mutans* in saliva and dental plaque during three-month follow-up period, concluded that NSF was more effective and beneficial than chlorhexidine and NaF. Other clinical trials proved the effectiveness of NSF when comparing it with a saline control and applied annually on primary [9] or permanent teeth [29].

Concerning the results of *in vitro* studies that evaluated the efficacy of NSF, the application of NSF on the surface of unaffected sound enamel was reported to be an effective caries preventive measure [30]. Some studies concluded that NSF might be more effective than

conventional fluoride preparations in treating incipient caries lesions [31, 32]. On the other hand, some *in vitro* studies showed that the remineralization effect of NSF was lower [33] or similar to the effect of NaF varnish [34], but the fluorine content of the NSF used in these *in vitro* studies was 10,147 ppm, while NaF varnishes contained 22,600 ppm of fluoride.

Regarding the n-HAP varnish, the results of the current study demonstrated that n-HAP had significantly lower efficacy than NSF and NaF in arresting initial lesions in 3-, 6-, and 12 months follow-ups. At the same time, there were no significant differences concerning preventive efficacy between the three preparations at 3- and 6-month follow-ups.

The effect of n-HAP could be explained by the action of small nanoparticles which fill in the surface irregularities of damaged enamel and produce a protective layer on the tooth's surface [8].

In the randomized controlled study by Najibfard *et al.* [35] 10% n-HAP dentifrice caused remineralization comparable to a 1100 ppm NaF toothpaste. Another clinical trial that evaluated the effectiveness of n-HAP paste and tri-calcium phosphate fluoride varnish revealed that n-HAP paste demonstrated better stability in its remineralization effect over the 6-month follow-up [36].

In the comparative study by Alhamed *et al.* [17] n-HAP gel turned out to be more effective than NaF varnish and tricalcium phosphate paste. An *in vitro* study proved that n-HAP brings effects similar to fluoride varnish and can be an alternative re-mineralizing agent for those at risk of dental fluorosis [37].

Differences in the results obtained in the cited research studies could be attributed to the use of different preparations, with different concentrations and application schedules.

The results of the present study revealed that the n-HAP varnish was less effective in preventing new caries lesions than NSF varnish at the 12-month follow-up and less effective than NSF and NaF varnishes in arresting the incipient lesions. It must be remembered that n-HAP can effectively prevent caries, hardening the enamel surface and arresting the existing initial caries due to its chemical and structural similarity to tooth mineral content. However, its action on bacteria is limited to reducing bacterial adhesion without killing the bacteria [38].

Thus, complex preparations containing both n-HAP and fluoride have been introduced. The study of Souza *et al.* [39] proved that n-HAP/fluoride toothpaste was the only one able to significantly reduce dentine demineralization and to improve enamel remineralization compared to placebo. MI Paste Plus (casein phosphopeptide-amorphous calcium phosphate, 0.2% NaF) and 0.2% NaF toothpaste were less effective.

The only study that has used the same three agents as our research was conducted in vitro to determine the re-mineralization ability of NaF varnish, n-HAP, and NSF on the enamel of primary anterior teeth. The study results showed that the greatest re-mineralization was observed in the NSF group [40], which is in agreement with our results.

The limitation of the study was that the process of blinding the dentist who applied the varnishes might be compromised because the color of the three agents was different as follows: n-HAP had a white color, NSF was reddish yellow, and NaF varnish was brownish. Moreover, fluoride varnish and n-HAP varnish were applied biannually, while NSF was used only once, so we could not evaluate the effects of the same schedule of applications. Further studies are required to assess long-term efficiency after 2-3 years when the regular application is discontinued.

CONCLUSIONS

Single application of the NSF varnish was significantly more effective than biannual application of n-HAP varnish. No significant differences were found between NaF and NSF groups, although a trend towards superiority of NSF varnish over NaF varnish in the prevention of new caries lesions was seen at the 12-month follow-up.

Based on the outcomes of this randomized clinical trial, annual application of NSF varnish might be recommended as an effective method of dental caries prevention and treatment of initial caries lesions.

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CONFLICT OF INTEREST

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