

Nanosilver Fluoride—A Paradigm Shift for Arrest in Dental Caries in Primary Teeth of Schoolchildren: A Randomized Controlled Clinical Trial

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ABSTRACT

Background: Due to limited financial resources, poor access to basic oral care, and the high cost of restorative treatment, children of low-income nations have their general health, social well-being, and education opportunities affected by untreated dental caries. Arresting caries treatment (ACT) has been proposed to manage untreated dental caries in children of disadvantaged communities.

Aims and objectives: The purpose of the present controlled clinical trial is to investigate the effectiveness of a new anticaries agent, nanosilver fluoride (NSF), preventing and arresting caries in children.

Materials and methods: A total of 100 deciduous molars both maxillary and mandibular are randomly selected from 60 children of 4–9 years of age group, which were randomly divided into NSF experimental group and saline control group. Teeth were clinically diagnosed and treated by one masked examiner and followed up at 7 days, 5 months and 12 months by another calibrated examiner who was blinded to the type of treatment. The criteria of the ICDAS II were followed to determine the activity of lesion and the diagnosis of caries. The Pearson's Chi-square test was used to compare the groups during different follow-up examinations.

Results: Seventy-eight percent of decayed teeth showed hard arrested dentine at 7 days; after 5-month analysis in the NSF group, 72.91% of the teeth showed arrested caries; and in the control group, only 34% of teeth showed arrest of caries. At 12-month analysis in the NSF group, 65.21% of teeth showed arrested cavities, and in the control group, 28.88% of teeth showed arrest of caries.

Conclusion: The present study proves that NSF is an anticaries agent and presents a noninvasive option for caries arrest and treatment when applied directly to dentin caries lesions.

Keywords: Caries arrest, Dental caries, Nanosilver fluoride.

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INTRODUCTION

The prevalence of dental caries is highly significant in children of lower socioeconomic status or lower income group; due to their socioeconomic status, treatment becomes unaffordable for them, and moreover, the lack of dental manpower and the sophisticated dental equipment required makes conventional restorative care a difficult way to solve the caries problem.¹

In deprived communities, alternative treatments for dental caries in children are very essential. Prevention and arresting or remineralization of carious lesions make caries easily treatable, affordable, and with the lowest use of equipment and ability to perform these treatments at community levels.¹

According to the present concept, the effect of fluoride on tooth decay control is attributed to its constant availability in the oral cavity, which remarkably helps by working directly on the process of demineralization/remineralization. In spite of their efficacy, application of topical fluoride is expensive, technique sensitive, and necessitates multiple applications in a year; therefore, alternative preparations provide further benefits may be of interest.²

Nanotechnology is increasingly regarded as a crucial emerging 21st century technology based on its economic and scientific potential and is experiencing rapid growth with many potential applications in dentistry.³ Due to its unique characteristics, silver nanoparticles (AgNPs) with a high specific surface area and a high fraction of surface atoms have attracted the attention.

Several studies indicate that the AgNPs antibacterial mechanism relies on their ability to penetrate the bacterial cell

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wall, resulting in direct and indirect lipid peroxidations which destroys the cell membrane, disrupts the replication of DNA, and restores and inhibits the respiratory protein.⁴ Silver diamine fluoride (SDF) has effectively prevented and halted coronary caries in primary teeth and also root caries in permanent teeth.² Moreover, adverse effects such as staining carious tissue black and reversible slightly painful lesions in oral mucosa caused by accidental contact with SDF solution have been documented by SDF. Nanosilver fluoride (NSF), a similar agent and a new experimental formulation containing AgNPs, chitosan, and fluoride, combines preventive and antimicrobial properties in

order to combat these drawbacks and has been developed as an effective anticaries agent.⁵

The present study was conducted due to the current limited research and scarcity of the literature on NSF. The aim of this controlled clinical trial is to investigate the efficacy of NSF, a new anticaries agent, preventing and arresting caries in children.

MATERIALS AND METHODS

Preparation of NSF⁵

To prepare the colloidal silver, 1.0 g of chitosan was dissolved in 200 mL of acetic acid 2% (V/V). The solution was stirred overnight and subsequently filtered under vacuum (Fig. 1A). Next, an aliquot of 60 mL of chitosan solution was placed in an ice bath while being stirred, and 4.0 mL was added to a solution of silver nitrate 0.012 mol L⁻¹ 30 minute before the addition of sodium borohydride (Fig. 1B). The relationship between AgNO₃ and NaBH₄ was maintained at a 1:6 mass and added dropwise. The reduction of Ag⁺ was initiated immediately, as the solution changed from colorless to light yellow and ended up reddish (Fig. 1C). The AgNPs had an average size of 3.2–1.2 nm and a spherical shape. 0.05 ppm of sodium fluoride (NaF) was added only at the end of the experiment, which improved the stability of the solution. The concentrations of each component, as expressed in micrograms per milliliter, were as follows: chitosan [28,585 µg/mL], Ag⁺ [376.5 µg/mL], and sodium fluoride [5028.3 µg/mL].

The present *in vivo* study was conducted in the Department of Pedodontics and Preventive Dentistry, Mamata Dental College and Hospital, Khammam, Telangana, from May 2016 to May 2017. Ethical clearance was obtained from Ethical Review Committee and Institutional Review Board of Mamata Dental College and Hospital. Prior to the study, written information explaining the purpose and the procedure of the study was explained and sent to the parents of the children involved in the study who sent back signed written informed consent, which was approved by head of the institution. A total of 100 deciduous molars both maxillary and mandibular are randomly selected from 60 children of 4–9 years of age group with a mean age of 6 years from the Grace Children School, Tanikella (Fig. 2), which is situated in eastern zone of rural Khammam, Telangana.

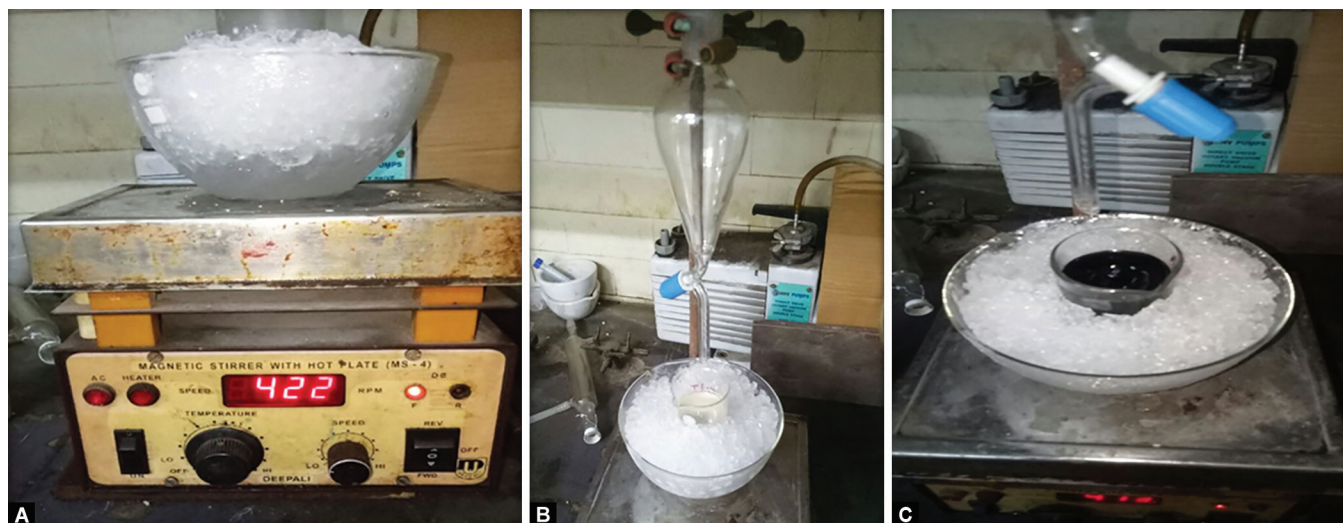
The drinking water supply was optimally fluoridated which is 0.9 ppm. All the children received similar diet, which was supplied by the institution authorities. All children participating in the study were provided with a fluoridated toothpaste (1000 ppm F) and were provided with oral hygiene and healthy diet instructions before their dental examinations.

This study design was a randomized, controlled, and double-blind trial. The teeth were selected and clinically treated by a single calibrated examiner. The follow-up exams were performed by another calibrated examiner who was blind to the type of treatment. The children and guardians were also blind to the type of treatment.

Inclusion criteria for the study were children of 4–9 years of age who had at least one primary tooth with active caries lesion corresponding to International Caries Detection and Assessment System II criteria code 05, where the tooth presents distinct cavity with visible dentin. Children were excluded from the study if they presented syndromes or were undergoing medical treatment for chronic or acute diseases, to avoid bias for reduced salivary flow and caries with pulpal exposure or fistula and swelling. At baseline, the children did not present caries in their permanent teeth.



Fig. 2: Grace Children School, Tanikella, Khammam, Telangana



Figs 1A to C: (A) Chitosan stirring overnight using magnetic stirrer; (B) Addition of AgNO₃ and NaBH₄; (C) Reduction of Ag⁺



Fig. 3: Application of nanosilver fluoride using microbrush



Fig. 4: Evaluation of arrested caries using CPITN probe during follow-up



Fig. 5: Arrested caries after 12 months

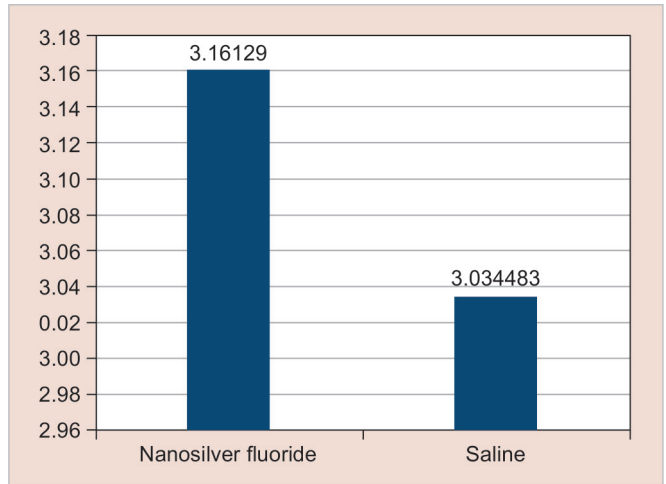


Fig. 6: Mean deft between experimental and control groups

A total of 300 school children were screened of which 240 children have not met the ICDAS II criteria. Among 60 children who were included in the present study, a total of 100 deciduous maxillary and mandibular molars with caries involving occlusal and smooth surfaces were randomly selected and divided into two groups of 50 teeth each. The method of randomization was performed to maintain a similar distribution of the number of teeth in each group.

Group I: Experimental group—NSF ($n = 50$).

Group II: Control group—saline ($n = 50$).

For caries treatment, the tooth was isolated from saliva with the help of cotton rolls and no effort was made to remove the caries or unsupported enamel. In experimental group, the selected tooth has received two drops of NSF (33,989.8 $\mu\text{g}/\text{mL}$) with a microbrush applicator tip, which is equivalent to a dose of 10 mg of the solution. For caries treatment in the control group, the selected tooth received two drops of saline with a microbrush applicator tip (Fig. 3). The NSF solution and saline were left in contact with the tooth surface for 2 minutes. Both treatments were performed only once in 12 months.

Follow-up

The follow-up assessment was done to the teeth of both the groups after 7 days, 5 months, and 12 months. The teeth were evaluated

clinically using visual and tactile inspection by a trained blind examiner who was blinded to the technique. The ICDAS II criteria were used to classify active caries lesions in both groups. Active caries was recorded with a blunt CPITN probe (Fig. 4), which when applied with light force, easily penetrated the dentine that where considered failure, whereas arrested caries (Fig. 5) was recorded and considered successful if the dentine could not be penetrated.

All the data after follow-up examination were tabulated to assess the success rate of the treatment with NSF and saline after 7 days, 5 months, and 12 months. The difference between the effects of two materials was statistically analyzed by Pearson's Chi-square test and $p < 0.005$ was considered statistically significant.

RESULTS

The present study comprised of 60 schoolchildren of mean age 6 ± 0.6 years; of these, 22 (36.6%) children were female and 38 (63.33%) children were male. All the teeth were primary posterior both maxilla and mandible and 66% of the carious lesions involved only one carious surface and 34% involved two or more surfaces.

The mean deft (decayed, extracted, and filled teeth) at the baseline was 3.08 ± 0.58 for both experimental group and control group with no statistical difference between the two groups (Fig. 6).

The number of teeth evaluated during the follow-up depicts that after one week from the baseline examination, there was no loss of the teeth in both the groups (Table 1). At 5 months, there were two losses of teeth in experimental group due to extraction and three in the control group. After 12 months, there were four losses in experimental group and five in control group of which three were due to change of school and others due to extractions.

Evaluation of the effectiveness of the treatments in the arrest of the decay (Table 2) (Fig. 7). After 7 days, analysis revealed that majority of the participants among NSF group, i.e., 78% of decayed teeth showed hard arrested dentine at 7 days and 22% of experimental group showed failure while all the participants in the control group showed no caries arrest on the 7th day of evaluation. After 5-month analysis in the NSF group, 72.91% of the teeth showed arrested caries, and in the control group, only 34% of teeth showed arrest of caries ($p < 0.001$). At 12-month analysis in the NSF group, 65.21% of teeth showed arrested cavities, and in

the control group, 28.88% of teeth showed arrest of caries, showing that majority of the participants among NSF group still retained caries arrest status after 12 months while some of the participants in the control group showed caries arrest; however, even after 12 months of evaluation, majority of them were still unarrested. This showed that the difference seen in the caries arrest phenomenon among both the groups still persisted, which was supported with a significant association between NSF and control groups with a p value of 0.003, indicating significant association between using NSF and caries arrest.

The evaluation of the effectiveness, according to the number of surfaces involved after 5 months (Table 3). In the NSF group among single surface, 75.75% of teeth showed success and 66.66% in multiple surface carious lesions ($p = 0.015$).

The evaluation of the effectiveness, according to the number of surfaces involved after 12 months (Table 4). In the NSF group, 67.74% teeth showed success in single surface carious lesions, and in the multiple surface carious lesions, 60% of teeth showed success showing that majority of the single surface carious lesions among NSF group still showed higher caries arrest status after 12 months while multiple surface showed less caries arrest. This showed that the difference seen in the caries arrest phenomenon between both the groups still persisted, which was supported with a significant association between single and multiple surface groups with a p value of 0.003.

Table 1: Number of teeth evaluated during follow-up

Group	n	7 days		5 months		12 months	
		n	Follow-up	n	Follow-up	n	Follow-up
NSF	50	50	48	50	48	50	46
saline	50	50	47	50	47	50	45

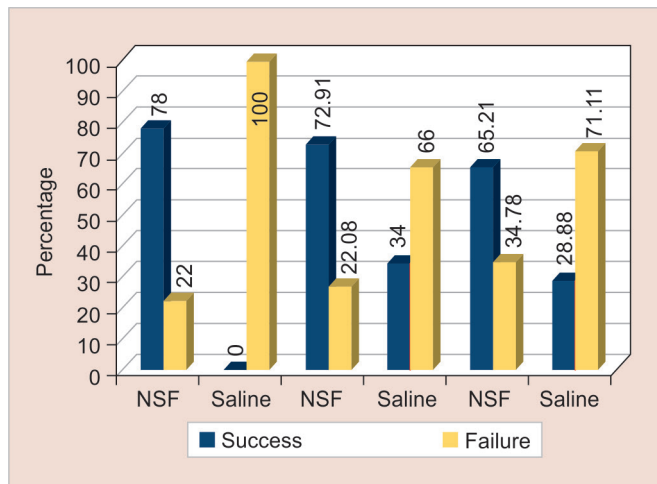


Fig. 7: Effectiveness of the treatments in the arrest of the decay

Table 2: Evaluation of the effectiveness of the treatments in the arrest of the decay

Variables	Groups				p value
	NSF		Saline		
	n	(%)	n	(%)	
7 days					
Success	39	78.0	0	0	<0.001
Failure	11	22.0	50	100	
5 months					
Success	35	72.91	17	34.0	<0.001
Failure	13	27.08	30	66.0	
12 months					
Success	30	65.21	13	28.88	0.003
Failure	16	34.78	32	71.11	

DISCUSSION

Dental caries is known as an infectious microbiological disease of calcified teeth tissues characterized by demineralization of inorganic portion and degradation of the tooth's organic substance.⁶ More than five *Streptococcus* species and *Actinomyces viscosus* are considered to be early colonizers of tooth surfaces, while Streptococci mutans such as *S. mutans* are considered. Sobry, *S. salivary*, *S. sanguinus*, and *S. mutans* are known to be effective dental biofilm colonizers. Plaque biofilm formation disruption is the key to successful dental caries management and prevention.⁷

Table 3: Evaluation of the effectiveness, according to the number of surfaces involved after 5 months

Treatment	Number of surfaces	5 months				Total	p value
		n	Success (%)	n	Failure (%)		
NSF	Single	25	75.75	8	24.24	33	<0.001
	Multiple	10	66.66	5	33.33		
Saline	Single	10	34.48	19	65.5	29	0.015
	Multiple	7	33.88	11	66.11		

Table 4: Evaluation of the effectiveness, according to the number of surfaces involved after 12 months

Treatment	Number of surfaces	12 months				Total	p value
		n	Success (%)	n	Failure (%)		
NSF	Single	21	67.74	10	32.25	31	0.003
	Multiple	9	60	6	40		
Saline	Single	8	28.50	20	71.40	28	0.045
	Multiple	5	26.41	12	73.58		

Due to extreme limited financial resources, inadequate access to basic oral care, and high cost of restorative therapy, children from low-income nations have their overall health, social well-being, and educational opportunities that are impaired by untreated dental caries. Arresting caries treatment (ACT) has been introduced to handle untreated dental caries in children from deprived communities.⁸ In the past decade, the use of nanotechnology over dentistry has received considerable attention. Nanoparticles have a wide range of pharmaceutical applications as their physical and chemical properties, such as shape, surface load, and hydrophobicity, can be modified to match their purpose. Nanoparticulate formulations can be administered as an aqueous solution for local applications in the oral cavity or formulated into a gel or paste producing products with high patient acceptance and easy administration. Nanoparticulate metals and metal oxides with bactericidal effects were of great interest concerning dental caries. Metal ions are active against these bacteria.⁹

Recently, a new formulation of NSF in combination with chitosan has been developed. The effectiveness of NSF in the arrest of caries can be demonstrated by the synergism of the components of its chitosan, AgNPs, and fluoride formulation.⁹ Silver is typically used in the form of nitrates to cause antimicrobial effects, but when AgNPs are used, the surface area available for exposure to the microbe is greatly increased.⁹ According to Sondi I, AgNPs are capable of anchoring and penetrating the bacterial cell wall, causing structural changes in the cell membrane such as cell membrane permeability and cell death. "Pits" are formed on the cell surface, and nanoparticles are deposited on the cell surface.¹⁰ Danilcauk and Kim¹¹ suggest that the creation of free radicals by AgNPs may be considered another process by which the cells die.

Studies of electron spin resonance spectroscopy have indicated that the AgNPs produce free radicals when in contact with the bacteria, and these free radicals can damage the cell membrane and make it porous, which can ultimately lead to cell death.

The potential agent used in the current caries arrest formulation is chitosan, a polysaccharide composed of glucosamine copolymers and *N*-acetyl glucosamine.³ Chemically, chitosan is generated through the regulated *N*-deacetylation of chitin, a linear polysaccharide derived primarily from arthropod exoskeletons such as marine crustaceans.

The resulting polycation is soluble in aqueous solutions of small organic acids such as acetic acid and lactic acids and can be linked in the presence of polyvalent anions such as phosphates.¹² During the adhesion process, chitosan can inhibit *Streptococcus mutans* and showed significant antibacterial and plaque reduction action at successive stages of accumulation so that chitosan is effective. Furthermore, *in vitro* studies have shown that chitosan interferes with demineralization of the tooth enamel inhibiting the release of mineral elements. In the present formulation, the mechanism of action of the third element fluoride is to prevent demineralization of enamel. Hiiri et al. supported the use of topical fluorides, as fluorides used in various forms have been shown to be active in the prevention of dental caries, may be a useful measure to avoid carious lesions.

Ten cate stated that fluoride absorbed by demineralized dental hard tissue along with calcium and phosphate is a crystalline structure (remineralization) that is more resistant to bacterial acid challenges. Fluoride has also been shown to inhibit the mechanism by which cariogenic bacteria metabolize carbohydrates to generate acids and thus affects adhesive polysaccharides bacterial growth.

So this present randomized, controlled, and double-blind clinical trial was planned to investigate the effectiveness of a new anticaries agent for preventing and arresting caries in children.

In the present study, ICDAS II¹³ criteria code 05 was taken as the inclusion criteria and also during evaluation in follow-up examination for assessing the caries activity. ICDAS is a method of diagnostic grading used for the diagnosis and evaluation of dental caries. It is developed for use in dental education, diagnostic procedures, epidemiological studies, and analysis. This scoring system can be used on coronal surfaces and root surfaces and can be used to identify and assess such lesions for enamel caries, dentine caries, and noncavitated lesions (as compared to many systems). Though depending on surface characteristics, ICDAS evaluates surface changes and potential histological depth of carious lesions. In ICDAS II criteria code 05, the number "0" corresponds to the carious surface which is not restored or sealed. Number "5" corresponds to a distinct cavity with visible dentin.¹⁴

In the present study, no effort was made to remove caries or unsupported enamel.

It is necessary to arrest caries without removing caries. First, the treatment methods will be much easier if caries is not excavated. Second, after obtaining basic practice, nondental workers may perform the procedure. Third, the noninvasive nature of the treatment makes subject adherence possible, even for young children.¹⁵ There is no prescribed rate of application of SDF solution in the dental literature. Horst et al. provided a detailed clinical approach in his article based on the findings of the systematic review.

For the first 2 years, they proposed the use of SDF twice a year for only carious lesions, without excavation. Any patient with active caries will obtain SDF instead of varnishing with sodium fluoride. Additional studies are needed when a routine application is withdrawn to assess long-term effectiveness after 2–3 years. According to the manufacturer's dose recommendations based on body weight, SDF must be used safely. The researchers set their prescribed limits per treatment visit at 1 drop per¹⁰ kilograms of body weight. It sets the short- and long-term requirements of the Environmental Protection Agency.¹⁶

According to Chu et al.,¹⁴ a 30-month study results showed that the annual application of SDF solution is effective in the arrest of dentine caries in primary anterior teeth in preschool Chinese children and Santos et al.⁵ also reported that single annual application of NSF may arrest dental caries. Application in this study has therefore been done only once in 12 months, and the present results indicate that NSF has been active when applied annually in arresting caries.

SDF's main advantage in preventing dentine caries is its efficacy. In most countries, it is inexpensive and therefore accessible. The process of treatment is quick and does not require expensive equipment or infrastructure support. It is noninvasive, and therefore, there is a very low risk of transmitting disease.

One of the disadvantages of using SDF to prevent caries is to stain the lesions black. The SDF solution also has a metallic taste on the oral mucosa and reversible white lesions when SDF comes into contact with the tissues. Mei et al.¹⁷ proposed that chemicals such as potassium iodide or AgNPs should be used to enhance the esthetic outcome and to overcome all SDF disadvantages. So, NSF was used in the present study which in contrary did not stain the teeth black and also children have not complained about the metallic taste of NSF and also no lesions were found on oral tissues.

Previous studies have found no evidence that fluorosis is caused by long-term application of SDF. However, after topical SDF therapy, the subjects made no complaints of discomfort. There was no leukoplakia in the gingiva or mucosa, and there was no swelling, pigmentation, or ulceration 24 hours after application of SDF. The NSF was not harmful at any concentration measured for any form of erythrocyte and is more biocompatible than the SDF.¹⁸ The 65.21% NSF-prevented fraction of caries arrested in the primary teeth was comparable to the NSF-preventive fraction reported in the Santos et al. clinical trial, which was 67% after 12 months and was also consistent with the NSF studies conducted by Llodra et al.¹⁹ with respect to the therapeutic effect of SDF (arrest of caries), with 77% success, both in deciduous teeth and in first permanent molars.

The results of the present study were lower than the outcome reported by Chu et al.,¹⁴ which was >96%, and by Zhi et al.,²⁰ where the caries arrest rate was significantly higher that is 91% when 38% SDF was applied semiannually and showed 79% arrest when 38% SDF applied annually. This can be explained by differing designs and as they have included only anterior tooth surfaces in the study design and also different application intervals.

According to the present study, control group has shown 34.7% of arrest after 12 months. The positive results of the control group can be explained considering the public water supply which was optimally fluoridated and also all the children in the study have received fluoridated toothpaste, dietary, and oral hygiene instructions.

In the present study, the single surface carious lesions have shown more arrest compared to the multiple surface carious lesions, and these single surface caries include buccal or lingual surface and multiple surfaces include proximal and occlusal surfaces. It can be because of the occlusal forces acting on the multiple surface lesions and these findings were in accordance with the study done by Yee et al.⁸ according to the number of surfaces involved and the study performed by Zhi et al. where single surface lesions have shown more arrest of caries.

In the present study, the single annual application of NSF has shown positive results of caries arrest and the results were not in agreement with Duangthip²¹ who compared the effectiveness of 30% SDF vs fluoride varnish in caries arrest rates. In his study, one group received SDF at baseline and again after 1 year, another group received 3 SDF applications at weekly intervals, and the third group received three applications of 5% NaF fluoride varnish at weekly intervals. After 18 months, the caries arrest rate of those receiving annual SDF application was significantly higher, i.e., 40% than group II with three applications of SDF or NaF varnish applications which were 35% and 27%, respectively. These results were comparatively less than present study which can be attributed to the follow-up of the study which is 18 months.

The present study has concluded that NSF can effectively arrest the carious lesion which was in accordance with Santos et al., Lo et al., and Chu et al. One of the studies by Santos found that 30% SDF was 1.73 times more effective to arrest caries than an interim restorative technique after 6 and 12 months. The present results were not in accordance by one study conducted by Monse²² who reported that SDF was less effective than atraumatic restorative treatment (ART) sealants; however, the trial design and follow-up showed deviations from the original protocol and also he has reported caries increments in toothbrushing and nontoothbrushing children treated with ART sealants or SDF as well as control groups.

CONCLUSION

Based on the findings of the present study, the following ensuing conclusion regarding NSF can be drawn:

- There were statistically significant differences at 12-month follow-up between NSF and the control to arrest caries at dentinal level in primary molars in children when applied annually.
- The present study proves that NSF is an anticaries agent and presents a noninvasive option for caries arrest and treatment when applied directly to dentin caries lesions.
- From a public health point of view, NSF might provide an option for treating patients with barriers to care.
- NSF application is an inexpensive, noninvasive, and nontoxic application and thus can be afforded by most communities; it can be safely used in children when two drops are applied annually. The treatment procedure is simple and requires no full dental equipment or a clinical setting and the risk of cross infection is significantly reduced.

Limitations

- Additional studies are needed to determine long-term effectiveness after 2–3 years when regular application is discontinued.
- Longer studies should be conducted with large sample size to evaluate the effectiveness of NSF as preventive and caries arresting agent.

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