ORIGINAL RESEARCH

A Comparative Evaluation of Nanosilver Fluoride, Chlorhexidine, and Sodium Fluoride When Used as a Varnish on *Streptococcus mutans* Levels in Children with Caries

Tinesh Raja[1], Nidhi Agarwal[2], Zohra Jabin[3], Ashish Anand[4], Nandita Waikhom[5], Vabool Thakur[6]

Abstract

Aim: The purpose of the present study is to evaluate the effect of nanosilver fluoride (NSF), chlorhexidine (CHX), and sodium fluoride (NaF) when used as a varnish on *Streptococcus mutans* levels in children with dental caries.

Study design: A total of 120 children (age range 8–12 years) with incipient caries were randomly assigned to four groups ($n = 30$): group I—NSF varnish, group II—CHX varnish, group III—NaF varnish, and group IV—control. Varnish application at baseline was performed once. To assess the levels of *S. mutans* using the culture method [colony-forming units (CFUs)] and optical density (OD), plaque and samples were taken at baseline (T0), 1 month (T1), and 3 months (T3). Additionally, the oral hygiene index-simplified (OHI-S) was noted for clinical assessment.

Results: By the end of 3 months, a statistically significant reduction in plaque CFU and salivary CFU was found in group II. At the conclusion of the 3 months, group I had the greatest decrease in OHI-S. After 3 months, the plaque CFU score did not differ significantly across groups I, II, and III. However, a statistically significant difference in OD values (p-value of 0.00) was discovered between group I and all other groups.

Conclusion: Children with early caries can effectively lower their *S. mutans* count by using NSF varnish.

Keywords: Chlorhexidine, Incipient caries, Nanosilver fluoride, Sodium fluoride, *Streptococcus mutans*, Varnish.

International Journal of Clinical Pediatric Dentistry (2024): 10.5005/jp-journals-10005-2831

Introduction

Dental caries remains the most common dental illness globally and is regarded a serious global health concern. According to the World Health Organization's 2003 oral health report, periodontal diseases and dental caries are pandemic ailments that affect the entire community, irrespective of age, gender, or socioeconomic status.[1] As preventive measures and community oral health care services are more challenging to access in developing nations like India, the issue is more concerning there. According to the 2002–2003 National Oral Health Survey, the decayed, missing, filled teeth (DMFT) index score for Indian children was approximately 2, and the prevalence of caries increased with age, rising from 51.9 to 63.1% in the 5–15-year-old age-group, respectively.[2]

Minimally invasive techniques to halt the progression of dental caries are replacing more traditional methods of treating the condition, which involves surgically removing the damaged dental tissue and then placing an appropriate restorative material. Remineralization aims to prevent dental cavities by comprehensively protecting the patient in the long run by intervening as soon as possible.

Sodium fluoride (NaF) varnish is one of the oldest and most widely used varnishes. It is professionally applied to the tooth surface, with four applications annually at weekly intervals to provide antimicrobial and anticary activity.[3] A cationic bisbiguanide with a broad antimicrobial range is chlorhexidine (CHX). In dentistry, it is widely used in a variety of forms, including dentifrices (0.4%), gels (1%), solutions (0.12 and 0.2%), and varnishes (1, 10, 20, and 35%). Compared to other applied agent forms, it has been proposed that the varnish form of CHX administration leads to a sustained reduction of *S. mutans*.[4]

[1]Department of Pediatric and Preventive Dentistry, Priyadarshini Dental College and Hospital, Tiruvallur, Tamil Nadu, India

[2,4–6]Department of Pediatric and Preventive Dentistry, Institute of Dental Studies and Technology, Ghaziabad, Uttar Pradesh, India

[3]Department of Pediatric Dentistry, College of Health, Medicine and Life Sciences, Brunel University, Uxbridge, London, United Kingdom

Corresponding Author: Tinesh Raja, Department of Pediatric and Preventive Dentistry, Priyadarshini Dental College and Hospital, Tiruvallur, Tamil Nadu, India, Phone: +91 8056297817, e-mail: tineshraja@gmail.com

How to cite this article: Raja T, Agarwal N, Jabin Z, *et al.* A Comparative Evaluation of Nanosilver Fluoride, Chlorhexidine, and Sodium Fluoride When Used as a Varnish on *Streptococcus mutans* Levels in Children with Caries. Int J Clin Pediatr Dent 2024;17(4):410–416.

Source of support: Nil

Conflict of interest: None

However, with advancements in technology, an innovative approach has been made to combat the high incidence of dental caries—the use of nanotechnology in dentistry. Silver nanoparticles, one of many types of nanomaterials, have demonstrated significant promise for use in biological applications. Many of the antibacterial effects of nanomaterials stem from the release of silver ions, which can pass through the cell wall of bacteria and cause both direct and indirect lipid peroxidation, damaging the cell membrane, halting deoxyribonucleic acid (DNA) replication, and affecting both respiratory protein restoration and inhibition.[5]

Thus, the objective of the current study was to discover how to varnish applications of NaF, CHX, and nanosilver fluoride (NSF) altered the levels of *S. mutans* in children with dental caries.

Materials and Methods

The study followed the Consolidated Standards of Reporting Trials (CONSORT) 2010 standards and was a randomized, triple-blinded clinical controlled trial (Fig. 1).

For every group (NSF, CHX, NaF, and control), a minimum sample size of 27 was advised, taking into account a power of 80% (1-β), with a 95% confidence interval and an effect size (*f*) of 0.39 using G*Power 3.1. An overall suggested sample size of 109 was estimated, which was rounded to 120 to ensure that each group had at least 30 samples.

A total number of 260 patients (age range of 8–12 years) were screened. Parental consent was obtained prior to their inclusion in the study. Cooperative children with fully erupted permanent central incisors and permanent first molars having incipient caries with International Caries Detection and Assessment System II (ICDAS II) scores 1 and 2 were included. Children having any intraoral hard or soft tissue infection or pulpally involved caries were excluded. Medically compromised children, or children on fluoride or antimicrobial therapy, and wearing orthodontic appliances were also excluded from the study. A total of 120 children were enrolled for the study on the grounds of pre-established eligibility criteria.

Examination Incipient Lesion

Visual examination was carried out to detect incipient lesions. The ICDAS II was used to standardize the diagnosis (Fig. 2).[6] Two investigators conducted the examination of the incipient lesion, and if there was any disagreement, the third investigator assessed the discrepancy and made the final decision.

To avoid selection bias, allocation concealment using the sequentially numbered, opaque, sealed envelopes approach was implemented. The random concealment was conducted by an investigator who was not engaged in the application of varnish or the measurement of the outcome to prevent intervention bias. An envelope with a dark color and a corresponding serial number on top was sealed with a sheet of paper bearing a randomized group number. After the intervention was allocated, the envelope was opened. The varnish was applied according to the group designated in the document.

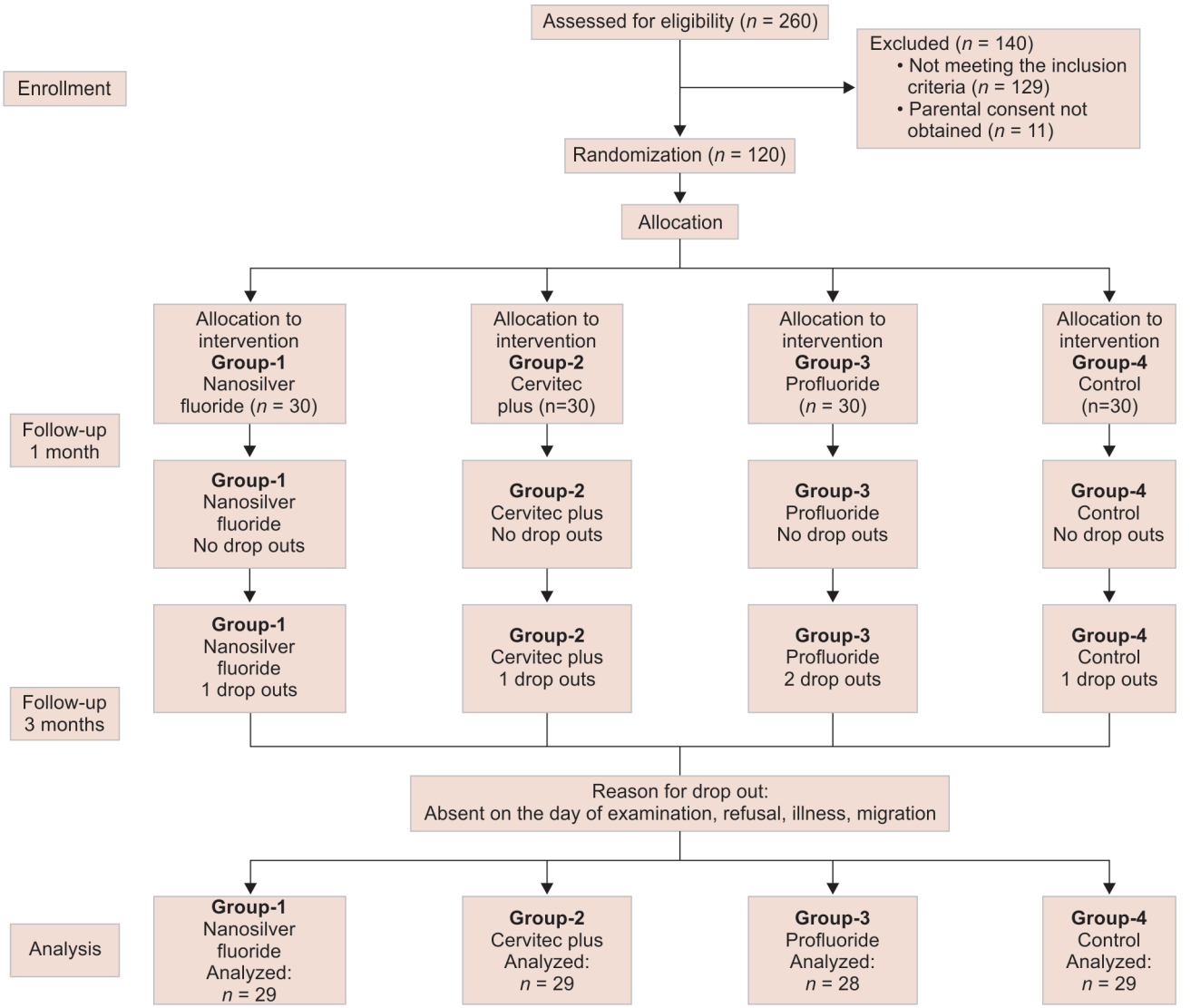

Fig. 1: Consolidated Standards of Reporting Trials flow diagram

The chosen research study participants were allocated randomly to one of four groups (30 children per group) (Table 1).

Preparation of Nanosilver Fluoride

The formulation provided by Targino et al. was followed in the preparation of NSF.[7] Silver nitrate was chemically reduced with sodium borohydride ($NaBH_4$) and chitosan biopolymer as a stabilizing agent to produce silver nanoparticles in an aqueous solution. We dissolved 2.5 mg/mL of chitosan in a 1% acetic acid solution. Magnetic stirring was used to combine the mixture until it was homogeneous. The mixture was then submerged in an ice bath, and it was vigorously stirred while more $NaBH_4$ (0.3 mL, 0.8 M) was added drop by drop. After removal from the cold bath, 10,147 parts per million of NaF were added to the flask. Stirring continued overnight. The resulting solution contained silver nanoparticles (399.33 µg/mL), NaF (10,147 µg/mL), and chitosan (2334 µg/mL). Transmission electron microscopy (TEM) was utilized to assess the shape and size of silver nanoparticles. A TEM image was captured on an FEI-Tecnai G2 F20 with an accelerating voltage of 200 kV. It was determined that 99% of the silver nanoparticles were spherical, with a size of 8 ± 2.0 nm (Fig. 3).

The child's personal information, dental history, and medical history, including any recent exposure to antibiotics, were documented prior to the study's start. During the trial period, food counseling and instructions on oral hygiene were provided. Evaluation was done for the following:

- Clinical parameter—oral hygiene index-simplified (OHI-S) index: The buccal surface of the index teeth—16, 11, 26, 36, 41, and 46—as well as the incisal two-thirds and cervical region were all traversed by an explorer. Based on the OHI-S, each of the six teeth received a score ranging from 0 to 3.[8]
- Method of dental plaque sample collection: Using a sterile wooden toothpick, the lingual and buccal surfaces of the index teeth—16, 11, 26, 31, and 46—from the occlusal to the gingival third were scraped to obtain plaque samples from each patient between 9 and 10 AM. After being collected, the samples were placed in a microcentrifuge tube (5 mL) with 3 mL of saline solution and sent directly to the lab.
- Method of saliva sample collection: Around 2–3 mm of unstimulated whole saliva was extracted from the child by instructing them to drool into a sterile container. The containers

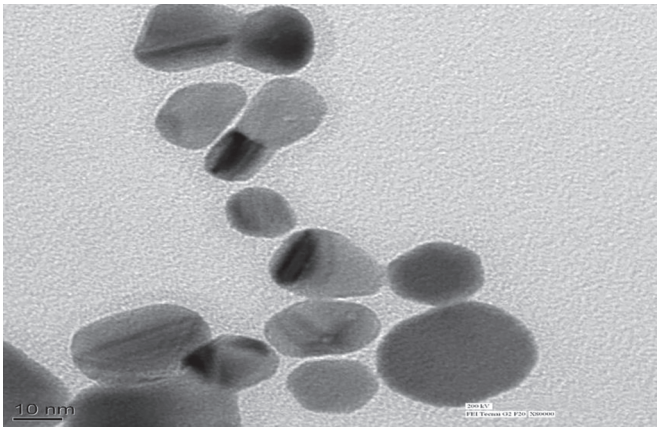

Fig. 3: Transmission electron microscopy image of nanosilver particles used in the study

CODE	DESCRIPTION
0	Sound tooth surface: No evidence of caries after 5 sec air-drying
1	First visual change in enamel: Opacity or discoloration (white or brown) is visible at the entrance to the pit or fissure seen after prolonged air drying
2	Distinct visual change in enamel visible when wet, lesion must be visible when dry
3	Localized enamel breakdown (without clinical visual signs of dentinal involvement) seen when wet and after prolonged drying
4	Underlying dark shadow from dentine
5	Distinct cavity with visible dentine
6	Extensive (more than half the surface) distinct cavity with visible dentine

Fig. 2: International Caries Detection and Assessment System II score and criteria

Table 1: Table showing group allocation and composition of materials used

Groups	*Number of participants*	*Materials*	*Composition*
Group I	30	NSF varnish	NSF group. A special formula as prepared by Targino et al.[7] containing nanosilver particles, NaF and chitosan as a stabilizer
Group II	30	CHX varnish	The CHX varnish (Cervitec Plus by Ivoclar Vivadent) group includes 1% CHX diacetate and 1% thymol as active antimicrobial ingredients
Group III	30	NaF varnish	The NaF (Profluoride Varnish, VOCO GmbH. Cuxhaven, Germany) varnish group contains 5% NaF (22,600 ppm F) with xylitol
Group IV	30	Control	Control group. Saline was used as a placebo control

were labeled and immediately submitted for analysis of *S. mutans*, then stored in a cold bath below 4°C.

Method of Varnish Application

Oral prophylaxis was performed to standardize the oral cavity with the least amount of biofilm before varnish was applied. The teeth were isolated using cotton rollers and a saliva ejector, and for 30 seconds, a three-way air syringe was used to gently inject air into the teeth. Using an applicator tip, approximately 0.1 mL of the chosen varnish was applied quadrant-wise sequentially to every tooth, beginning with the upper arch.

Participants were instructed not to rinse, eat, or drink anything for 3 hours and not to brush until the next day after application. Additionally, toothpaste devoid of fluoride was provided for use during the study.

Laboratory Phase

Two methods were used to evaluate *S. mutans* in plaque and saliva samples:

- Culture test: Mitis salivarius bacitracin agar was prepared to facilitate the multiplication and selection of *S. mutans* on the agar plates. Before distributing the saliva and plaque samples onto the agar plates for colony counting, they were diluted 1:1 and 1:2, respectively. The Petri plate was divided into eight sections, and the number of colonies found in each area was tallied. This number was then multiplied by eight to determine the total number of colonies on the plate. To acquire the total number of colonies in 1 mm of saliva or plaque, multiply the number of colonies obtained by the dilution factor.
- Optical density (OD): At 600 nm, OD was measured using a spectrophotometer (Spectronic 20). The OD method, based on the scattering of light, is used to track the kinetics of growth.

After applying varnish, the clinical and laboratory parameters were evaluated at baseline (T = 0), 1 month (T = 1), and 3 months (T = 3). A total of 5 of the 115 children in the final study sample had dropped out after 3 months (Fig. 1).

Results

A *post hoc* Bonferroni test and a one-way analysis of variance (ANOVA) were used to compare the *S. mutans* count at different intervals according to the application of different varnishes. During the follow-up phase, five participants withdrew from the original sample of 120 subjects, leaving 115 children, ages 8–12, remaining in the study. Of these, 53 boys (46.08%) and 62 girls (53.91%) made up the final study sample after 3 months.

Oral hygiene index-simplified: Each group's mean baseline OHI-S fell into one of four categories—0.4 ± 0.1, 0.5 ± 0.1, 0.5 ± 0.1, and 0.5 ± 0.2. All groups exhibited an increase in their OHI-S score, but group I saw the largest increase after 3 months, measuring 0.30 ± 0.15, which was determined to be statistically significant (Table 2).

Plaque: The four groups had baseline plaque colony-forming units (CFUs) of $4.48 \pm 1.34 \times 10^5$, $4.69 \pm 1.65 \times 10^5$, $4.41 \pm 1.50 \times 10^5$, and $4.57 \pm 1.34 \times 10^5$ CFU/mL, respectively. Group II showed the greatest decrease in plaque CFU score one month following the application of varnish, followed by groups III, I, and IV. Group II saw the greatest reduction after 3 months. Upon analysis, the decrease was found to be statistically significant in all groups with the exception of the control group.

The baseline plaque OD for all the groups was 1.38 ± 0.26, 1.39 ± 0.21, 1.44 ± 0.20, and 1.36 ± 0.17 OD/mL. Group I had the greatest decrease in plaque OD score one month following varnish application, followed by groups II, III, and IV. Group I showed the greatest decline after 3 months. With the exception of the control group, the decrease was statistically significant in every group (Table 2).

After 3 months, the intergroup comparison revealed that there was no statistically significant difference (p-value < 0.01) in the plaque CFU scores between groups I, II, and III. On the other hand, a statistically significant difference (p-value = 0.00) in OD was observed between group I and the remaining groups.

Saliva: The baseline salivary CFUs for all the groups were $4.43 \pm 1.35 \times 10^5$, $4.55 \pm 1.65 \times 10^5$, $4.31 \pm 1.57 \times 10^5$, and $4.05 \pm 1.61 \times 10^5$ CFU/mL. After 3 months, there was a statistically

Table 2: Table showing mean OHI-S, plaque CFU, and OD values recorded for groups I, II, III and IV at baseline (T0), 1 month (T1) and 3 months (T3)

		T0		*T1*		*T3*			
	Parameters	*Mean*	*Standard deviation (SD)*	*Mean*	*SD*	*Mean*	*SD*	*Mean change from T0 to T3*	*p-value*
Group I	OHI-S†	0.4	0.1	0.28	0.13	0.30	0.15	0.12	0.003*
	CFUP‡ (×10⁵ CFU/mL)	4.48	1.34	0.00556	0.00632	0.03115	0.01797	4.45	0.017*
	ODP§ OD/mL	1.38	0.26	0.463	0.181	0.400	0.125	0.98	0.000*
Group II	OHI-S†	0.5	0.1	0.38	0.12	0.43	0.13	0.07	0.113
	CFUP‡ (×10⁵ CFU/mL)	4.69	1.65	0.00315	0.00213	0.02634	0.01151	4.66	0.017*
	ODP§ OD/mL	1.39	0.21	0.888	0.153	0.785	0.169	0.61	0.001*
Group III	OHI-S†	0.5	0.2	0.41	0.14	0.39	0.14	0.08	0.128
	CFUP‡ (×10⁵ CFU/mL)	4.41	1.50	0.00439	0.00504	0.03405	0.01962	4.37	0.001*
	ODP§ OD/mL	1.44	0.20	0.932	0.177	0.913	0.223	0.52	0.000*
Group IV	OHI-S†	0.5	0.2	0.51	0.15	0.49	0.14	0.02800	0.541
	CFUP‡ (×10⁵ CFU/mL)	4.57	1.34	4.26033	1.20203	4.33133	1.23614	0.23533333	0.090
	ODP§ OD/mL	1.36	0.17	1.096	0.105	1.102	0.087	0.259333	0.092

One-way ANOVA applied; *, p-value significant at $p < 0.05$; OHI-S†, oral hygiene index-simplified; CFUP‡, colony forming unit in plaque; ODP§, plaque optical density

significant difference in the *S. mutans* levels for each of the three experimental groups, as determined by the OD and the culture technique. After 1 month, both groups I and II showed the greatest reduction. Group II had the greatest reduction after 3 months (Table 3). After 3 months, the intergroup comparison revealed no discernible differences between groups I, II, and III (Table 4).

An analysis using OD revealed a highly significant difference (*p*-value 0.00) between groups I and II, II and III, and III and IV.

Discussion

The results of the current study demonstrate that after 3 months, a single application of NaF, CHX, and NSF varnish remarkably reduced the amount of *S. mutans* in saliva and plaque in children with dental caries. The etiological factor, which includes host factors, diet, and dental plaque (*S. mutans*), is the most significant risk factor for any disease. In 1980, Hamada and Slade implicated *S. mutans*) as a primary causative organism of dental caries.[9] Hence, to control the cariogenic activity, it is important to suppress the growth of *S. mutans* counts in the oral cavity. The initial stage of tooth decay or demineralization is represented by the incipient carious lesions, which have the potential to progress to cavitation, be arrested, or reversed.

This study adopted a nonintensive varnish application regime, applying the coating once at baseline, which is in line with research conducted by Ben Khadra et al. and Al-Jaradi et al.[4,10] Despite using a more rigorous application regimen in their trial, Twetman et al.

Table 3: Table showing mean saliva CFU and OD values recorded for groups I, II, III and IV at baseline (T0), 1 month (T1) and 3 months (T3)

		T0		*T1*		*T3*		*Mean change*	
	Parameters	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*from T0 to T3*	*p-value*
Group I	CFUS† (×10^5 CFU/mL)	4.43	1.35	0.00463	0.00368	0.0500	0.0559	4.38	0.044*
	ODS‡ OD/mL	1.40	0.24	0.476	0.164	0.415	0.127	0.98	0.001*
Group II	CFUS† (×10^5 CFU/mL)	4.55	1.65	0.00323	0.00199	0.0264	0.0127	4.53	0.014*
	ODS‡ OD/mL	1.39	0.20	0.887	0.168	0.781	0.172	0.61	0.001*
Group III	CFUS† (×10^5 CFU/mL)	4.31	1.57	0.00938	0.01950	0.0804	0.1133	4.23	0.007*
	ODS‡ OD/mL	1.44	0.19	0.941	0.187	0.916	0.217	0.52	0.000*
Group IV	CFUS† (×10^5 CFU/mL)	4.05	1.61	4.18333	1.59155	4.1460	1.5723	−0.0970	0.070
	ODS‡ OD/mL	1.36	0.20	1.154	0.094	1.141	0.091	0.223333	0.068

One-way ANOVA applied; *, *p*-value significant at $p < 0.05$; CFUS†, colony forming unit in saliva; ODS‡, saliva optical density

Table 4: Intergroup comparison of CFU and OD among the four groups at 3 months

	Group (I)	*Group (J)*	*Mean difference (I–J)*	*p-value*
CFUP† (×10^5 CFU/mL)	I	II	0.00481067	1.00
		III	−0.00290333	1.00
		IV	−4.30018333*	0.000*
	II	III	0.00771400	1.00
		IV	−4.29728000*	0.000*
	III	IV	−4.30499400*	0.000*
ODP‡ OD/mL	I	II	−0.384400*	0.000*
		III	−0.512200*	0.000*
		IV	−0.701333*	0.000*
	II	III	−0.127800*	0.014*
		IV	−0.316933*	0.000*
	III	IV	−0.189133*	0.000*
CFUS§ (×10^5 CFU/mL)	I	II	0.0236033	1.00
		III	−0.0304533	1.00
		IV	−4.0960400*	0.000*
	II	III	0.0540567	1.00
		IV	−4.0655867*	0.000*
	III	IV	−4.1196433*	0.000*
ODS¶ OD/mL	I	II	−0.366000*	0.000*
		III	−0.500833*	0.000*
		IV	−0.725667*	0.000*
	II	III	−0.134833*	0.008*
		IV	−0.359667*	0.000*
	III	IV	−0.224833*	0.000*

Post hoc Bonferroni applied; *, *p*-value significant at $p < 0.05$; CFUP†, colony forming unit in plaque; ODP‡, plaque optical density; CFUS§, colony forming unit in saliva; ODS¶, saliva optical density

found that this did not increase the varnish's efficacy. Even after 1 month, the single treatment regimen used in this investigation significantly reduced the amount of *S. mutans*.[11]

Various literature on evaluating the presence of *S. mutans* have recommended saliva as a suitable method for predicting caries activity and identifying patients with high-risk of dental caries.[12] Gibbons and Houte, in 1975, stated that plaque is more appropriate and superior than saliva for estimating MS in individuals because tooth surfaces are the natural habitat of MS and Plaque.[13] Therefore, in the current study, we analyzed *S. mutans* levels in both saliva and plaque samples.

A novel varnish called NSF combines nanosilver particles with fluoride and chitosan to act as a stabilizing agent. Free radicals produced by the silver nanoparticles harm the bacterial cell membrane, cause it to become porous, and ultimately cause cell death. Moreover, during protein synthesis, silver ions can bind with sulfuryl groups and obstruct DNA replication.[14,15]

Nanosilver fluoride is endowed with remineralizing characteristics by the fluoride it contains, which lowers adhesion and biofilm formation.[16,17] As a result, NSF functions as a remineralizing agent in addition to having an antibacterial impact.

Besinis et al. in 2014 compared the antibacterial effect of silver nanoparticles with CHX and found that the antibacterial activity measured in terms of CFUs of silver nanoparticles was 25-fold higher than CHX.[18]

In 2017, Soekanto et al. assessed the effectiveness of NSF in preventing the production of *S. mutans* biofilms *in vitro*. They discovered that NSF was a more effective inhibitor of *S. mutans* biofilm formation than the industry standard silver diamine fluoride (SDF) (38%).[19]

El-Desouky et al. 2020 evaluated the anticarcinogenic effects of NSF and NaF in an *in vitro* study where the difference between *S. mutans* values in both the groups within a period of 7 days was found to be nonsignificant. Their result is in contrast to the present study since NSF has shown a significantly greater reduction in *S. mutans* count than NaF at the end of 1 and 3 months.[20]

A study by Waikhom et al. in 2022 found that when children without dental cavities applied NSF varnish, the number of *S. mutans* in both plaque and saliva decreased statistically significantly.[21]

In the present study, the CFU count of *S. mutans* in saliva and plaque did not show any statistically significant difference between NSF, CHX and NaF varnish. However, the reduction was significantly greater in NSF when the evaluation was done using the OD method. Since OD measurement is related to changes in morphology, clumping, or formation of long chains of bacteria during growth, it can be assumed that NSF causes some alteration in bacterial growth. The same can be appreciated intraorally by the significant reduction in values of the OHI-S index of the subjects where NSF varnish was used.

According to us, the potential limitation could be that this study was based on NSF varnish concentration, which was prepared by Targino et al. and is not a standardized concentration. However, more studies to standardize the concentration of NSF need to be carried out in future.

Conclusion

The present trial findings depicted that NSF containing remineralizing efficiency of fluoride and antimicrobial activity of nanosilver particles is effective in the reduction of *S. mutans* count in children with incipient caries.

Highlights of Our Study

- This paper acknowledges the use of NSF varnish in reducing *S. mutans* counts in children with caries.
- Nanosilver fluoride is a noninvasive agent that is highly safe for use in younger children.
- Nanosilver fluoride can be considered an alternative treatment modality owing to its promising caries reduction potential, as fluoride in NSF also provides remineralizing potential.

Ethics

Approval for this study was obtained from the local Institutional Review Board (Institute of Dental Studies and Technology, Ghaziabad, Uttar Pradesh, India) Ref. number: IDST/IEC/2020-23/19.

Orcid

Tinesh Raja https://orcid.org/0000-0002-1226-0470
Nidhi Agarwal https://orcid.org/0000-0002-9231-8256
Ashish Anand https://orcid.org/0000-0002-9813-5987
Vabool Thakur https://orcid.org/0000-0002-1313-2075

References

1. Petersen PE. The world oral health report 2003: continuous improvement of oral health in the 21st century – the approach of the WHO global oral health programme. Community Dent Oral Epidemiol 2003;31(Suppl 1):3–23. DOI: 10.1046/j..2003.com122.x
2. Bali RK, Mathur VB, Talwar PP, et al. National Oral Health Survey and Fluoride Mapping. New Delhi: Dental Council of India; 2002.
3. Knutson JW, Armstrong WD. The effect of topically applied sodiu fluride on dental caries experience. Public Health Rep (1896) 1970;58(47):1701.
4. Ben Khadra GM, Arrag EA, Alammori M, et al. The effect of chlorhexidine-thymol and fluoride varnishes on the levels of Streptococcus mutans in saliva in children aged 6–8 years. Indian J Dent Res 2019;30(1):67–72. DOI: 10.4103/ijdr.IJDR_208_17
5. Yin IX, Zhao IS, Lei M, et al. Use of silver nanomaterials for caries prevention: a concise review. Int J Nanomed 2020;15:3181–3191. DOI: 10.2147/IJN.S253833
6. Gugnani N, Pandit IK, Srivastava N, et al. International caries detection and assessment system (ICDAS): a new concept. Int J Clin Pediatr Dent 2011;4(2):93–100. DOI: 10.5005/jp-journals-10005-1089
7. Targino AGR, Flores MA, dos Santos Junior VE, et al. An innovative approach to treating dental decay in children. A new anti-caries agent. J Mater Sci Mater Med 2014;25(8):2041–2047. DOI: 10.1007/s10856-014-5221-5
8. Greene JC, Vermillion JR. The simplified oral hygiene index. J Am Dent Assoc 1964;68:7–13. DOI: 10.14219/jada.archive.1964.0034
9. Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev 1980;44(2):331–384. DOI: 10.1128/mr.44.2.331-384.1980
10. Al-Jaradi AM, Hamdy M, Ali W. Clinical evaluation of the anti-bacterial effect of fluoride and chlorhexidine varnishes. Egyptian Dent J 2013;59(7):2489–2501.
11. Twetman S, Petersson LG, Pakhomov GN. Caries incidence in relation to salivary mutans streptococci and fluoride varnish applications in preschool children from low- and optimal-fluoride areas. Caries Res 1996;30(5):347–353. DOI: 10.1159/000262341
12. Damle SG, Loomba A, Dhindsa A, et al. Correlation between dental caries experience and mutans streptococci counts by microbial and molecular (polymerase chain reaction) assay using saliva as microbial risk indicator. Dent Res J (Isfahan) 2016;13(6):552–559. DOI: 10.4103/1735-3327.197035
13. Gibbons RJ, Houte JV. Bacterial adherence in oral microbial ecology. 1975;29(1):19–42. DOI: 10.1146/annurev.mi.29.100175.000315

14. Kim JS, Kuk E, Yu KN, et al. Antimicrobial effects of silver nanoparticles. Nanomedicine 2007;3(1):95–101. DOI: 10.1016/j.nano.2006.12.001
15. Haghgoo R, Saderi H, Eskandari M, et al. Evaluation of the antimicrobial effect of conventional and nanosilver-containing varnishes on oral streptococci. J Dent 2014;15(2):57–62.
16. Silva AVC, Teixeira JA, de Melo Júnior PC, et al. Remineralizing potential of nano-silver-fluoride for tooth enamel: an optical coherence tomography analysis. Brazilian Res Pediatr Dent Integr Clin 2019;19. DOI: 10.4034/PBOCI.2019.191.50
17. Nanda KJ, Naik S. An in-vitro comparative evaluation of pre-treatment with nano-silver fluoride on inhibiting secondary caries at tooth restoration interface. Cureus 2020;12(5):e7934–e7939. DOI: 10.7759/cureus.7934
18. Besinis A, De Peralta T, Handy RD. The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. Nanotoxicology 2014;8(1):1–16. DOI: 10.3109/17435390.2012.742935
19. Soekanto SA, Marpaung LJ, Djais A, et al. Efficacy of propolis fluoride and nano silver fluoride for inhibition of streptococcus mutans and enterococcus faecalis biofilm formation. Int J Appl Pharmaceut 2017;9(2):51–54. DOI: 10.22159/ijap.2017.v9s2.13
20. El-Desouky D, Hanno A, Dowidar K, et al. Evaluation of the anticariogenic effect of nano silver fluoride on demineralization of enamel in primary teeth (an in vitro study). Alexandr Dent J 2020;46(2):153–159. DOI: 10.21608/adjalexu.2020.20537.1017
21. Waikhom N, Agarwal N, Jabin Z, et al. Antimicrobial effectiveness of nano silver fluoride varnish in reducing Streptococcus mutans in saliva and plaque biofilm when compared with chlorhexidine and sodium fluoride varnishes. J Clin Exp Dent 2022;14(4):e321–e328. DOI: 10.4317/jced.59093