EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 81:87, JANUARY, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORTHODONTICS, PEDIATRIC AND PREVENTIVE DENTISTRY
Submit Date : 01-11-2024 • Accept Date : 14-11-2024 • Available online: 1-1-2025 • DOI : 10.21608/edj.2024.332848.3249

EVALUATION OF THE STAINING POTENTIAL OF SILVER DIAMINE FLUORIDE AND NANO SILVER FLUORIDE ON PRIMARY TEETH: AN IN VITRO STUDY

Hebatalrahman Yehia Kishk*, Mina Kamal Yassa**
and Ahmad Abdel Hamid ELheeny***

ABSTRACT

Background: Despite the extensive literature validating the efficacy of Nanosilver fluoride (NSF) as a cariostatic agent, limited information exists regarding its surface staining potential. **Aim:** In vitro evaluation of surface staining changes over time of demineralized enamel of primary teeth treated with silver diamine fluoride (SDF), SDF and potassium iodide (KI), or NSF using VITA Easy Shade digital spectrophotometer, before treatment, one hour, two- and four weeks after treatment. **Results:** spectrophotometric color analysis showed that SDF-treated demineralized enamel exhibited the highest staining compared to either NSF or SDF+KI at the 3 time intervals and the results were statistically significant ($p < 0.001$). Comparing the staining potential between the SDF+KI and NSF groups, similar staining values were observed one hour after application. However, $\Delta E2$, $\Delta L2$, $\Delta E3$, and $\Delta L3$ values indicated the less staining potential of NSF on enamel surfaces after 2 and 4 weeks of application, however, results were only statistically significant for $\Delta L2$ ($p= 0.005$). **Conclusion:** NSF could be a more esthetic alternative to SDF, and SDF+KI when applied to demineralized enamel of primary teeth.

KEYWORDS: Silver diamine fluoride, Nano silver fluoride, Potassium iodide, Primary teeth.

INTRODUCTION

Dental caries is one of the most common chronic diseases [(1)]. **Recently, caries management has shifted to** minimal intervention dentistry (MID) which offers a more conservative treatment by managing and preventing carious lesions in their early stages without removing hard dental tissues.

MID frequently uses fluoridated and antimicrobial agents. The caries-arresting properties of silver diamine fluoride (SDF) have gained considerable

* Dentist at Assiut University Hospitals, Postgraduate Student At Pediatric and Community Dentistry Department, Faculty of Dentistry, Minia University, Egypt.
** Lecturer in Paediatric and Community Dentistry Department, Faculty of Dentistry, Minia University, Minia, Egypt.
*** Assistant Professor in Pediatric and Community Dentistry Department, Faculty of Dentistry, Minia University, Minia, Egypt.

attention. It is effective against cariogenic bacteria and is capable of remineralizing dentin and enamel[2]. However, the black stains that remain after application to carious dental tissues influence patients' and parents' acceptance of this treatment[3]. Aiming to overcome the black discoloration of SDF, potassium iodide (KI) was used in conjunction with it[4]. However, the arrested enamel lesions treated with this combination still showed perceptible discoloration[5].

The recently introduced Nano silver compounds, such as Nano silver fluoride (NSF), are proposed to be more aesthetic cariostatic agents. The NSF application is safe for both adults and children to use, noninvasive, nontoxic, and reasonably priced [6]. It is claimed to cause less discoloration or irritation to the soft tissues than SDF. It possesses the antibacterial actions of Silver Nanoparticles (AgNPs) and remineralizing properties of fluoride [7-9].

Despite the extensive literature validating the efficacy of Nanosilver fluoride (NSF) as a cariostatic agent [8-11], very limited information exists regarding its surface staining potential. Asghar et al. (2023) [12] systematically reviewed the literature regarding the efficacy of different approaches for minimizing SDF– mediated tooth discoloration, they found only one study that was performed by Espíndola et al. (2020) [7] which assessed the staining potential of NSF on dentin of permanent teeth.

Accordingly, and up to our knowledge, this is the first in-vitro study comparing the staining effect of SDF, SDF+KI, and NSF on demineralized enamel of primary teeth using VITA Easy Shade digital spectrophotometer, before treatment, one hour, two- and four weeks after treatment.

MATERIALS AND METHODS

An in vitro study was designed to evaluate the staining potential of SDF, SDF+KI, and NSF on primary teeth after one hour, two weeks, and four weeks of application of these cariostatic agents. The study was approved by the Ethics Committee of the Faculty of Dentistry, Minia University (Research code: 558 /2022).

Thirty extracted sound human primary teeth were collected from the outpatient clinic of the pediatric and community dentistry department at Minia University Dental Hospital. Extraction was indicated for one of the following reasons: prolonged retention or orthodontic purposes. The children's legal guardians permitted using their extracted teeth for research purposes. Sound primary teeth without cracks, fractures, caries, developmental anomalies, or restorations, were selected for the study.

Sample preparation

Selected teeth were cleaned with a prophylactic brush and washed with running water. Then, they were stored in distilled water and thymol until the time of the experiment. Circular adhesive tapes of 6 mm diameter were applied on the tooth surface. The remaining tooth surface was coated with an acid-resistant nail varnish. After drying the nail varnish, the adhesive tapes were removed leaving a circular window without nail varnish.

Teeth immersion in a demineralizing solution containing 2.2mM CaCl2, 2.2mM KH2PO4, 0.05mM Acetic acid, and pH adjusted to 4.4 1M KOH [13,14] was performed over seven days. Then, each tooth was mounted separately on an acrylic block. After being numbered, the thirty samples were randomly divided into three groups (n = 10 per group). Group (1) teeth received SDF treatment (control group); group (2) teeth received (SDF+KI) treatment, and group (3) teeth received NSF treatment.

Cariostatic Agents

38% **SDF** (TOOTHMATE ™, Egypt) and 30% **KI** (TOOTHMATE ™, Egypt) were purchased while **NSF** in a liquid form (1000 ppm) was laboratory prepared (Nanotech for Photo-Electronics, Cairo - Egypt).

NSF preparation: Using a magnetic stirrer, chitosan (28.7 ml, 2.5 mg/ml) was first dissolved in 1% acetic acid overnight. The mixture was then put into a flask and transferred to an ice-cold bath after being filtered through a vacuum filter unit. Then the mixture was stirred vigorously before adding 1 ml of silver nitrate (0.11 mol/L). Afterwards, a drop-by-drop, freshly prepared sodium borohydride (0.3ml, 0.8mol/L) was added. Silver (Ag+) was reduced instantly and the solution turned from colorless to pale yellow and finally dark yellow. Sodium fluoride (10,147 parts per million) was added, after taking the flask out of the ice bath[7]. The prepared NSF was characterized using a Transmission Electron Microscope (Figure 1).

Cariostatic agent application

Agents were applied onto the exposed demineralized enamel lesions with a micro brush. Group (1) teeth were treated with 38% SDF for two minutes before rinsing with distilled water. Group (2) teeth were treated with SDF and KI right after the SDF was applied. KI was applied with a microbrush to the same location until the reactionary white precipitate became transparent. Using a microbrush, each tooth in group (3) received two drops of NSF solution applied to the exposed enamel window. After two minutes of contact with the solution, the tooth surface was rinsed with distilled water.

Color Measurement

Teeth color was measured using a digital spectrophotometer (VITA Easyshade, VITA Zahnfabrik, Bad Säckingen, Germany) at 4 different time points (before agent application, then 1 hour, 2- and 4 weeks after application). Tooth staining analyzation was done based on the CIELAB color space and expressed as ΔE and ΔL. Prior to each color measurement, calibration of the spectrophotometer was performed [15-17].

The dental staining was determined using the CIELAB system parameters, which are (L*a*b*), where L* stands for lightness and the mean value ranges from 0 (black) to 100 (white). The values of a* and b*, respectively, represent the red-green and yellow-blue axes. The total color differences (ΔE) of enamel staining were computed using the following formula[7,18,19]. $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$.

In each separate group, the difference between baseline and after one hour (ΔE1 and ΔL1) were compared with those of baseline- two weeks (ΔE2 and ΔL2) and baseline- four weeks (ΔE3 and ΔL3).

Statistical analysis

SPSS (Statistical Package for the Social Science, version 26, IBM, and Armonk, New York) was used to analyze the collected data. To determine data compliance with normal distribution the Shapiro test was used. Quantitative data were expressed as mean

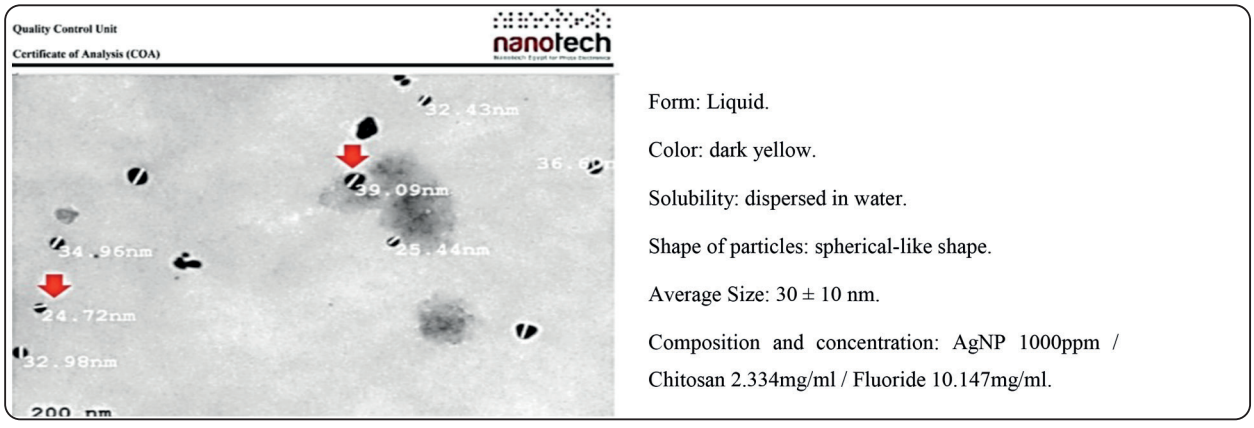

Fig. (1) Transmission Electron Microscope micrograph of NSF solution immediately after preparation. It shows predominantly spherical silver nanoparticles (arrow) dispersed in the colloidal solution.

and standard deviation (SD) and compared with the ANOVA test followed by post-hoc analysis. In each group, the difference between baseline and after one hour (ΔE1 and ΔL1) were compared with those of baseline-two weeks (ΔE2 and ΔL2) and baseline-four weeks (ΔE3 and ΔL3) by paired t-test. The confidence level was kept at 95%; hence, p-value <0.05 was considered significant.

RESULTS

In each group, ΔL values indicated that enamel lesions got darker with time. A similar finding was observed for ΔE values for SDF and NSF groups while for the SDF+KI treated lesions, ΔE2 was higher than ΔE3.

SDF-treated demineralized enamel exhibited the highest staining compared to either NSF or SDF+KI at the 3 time intervals and the results were statistically significant ($p < 0.001$).

Comparing the staining potential between the SDF+KI and NSF groups, similar staining values were observed one hour after application. However, ΔE2, ΔL2, ΔE3, and ΔL3 values indicated less staining effect of NSF on enamel surfaces after 2 and 4 weeks of application, compared to SDF+KI. The results were only statistically significant for ΔL2 ($p= 0.005$) (Table: 1, Figure 2).

TABLE (1) Enamel color changes of ΔE and ΔL after 1 hour, 2 weeks and 4 weeks of application of cariostatic agents in the three groups

	ΔE1 (Baseline-after one hour)	**ΔL1 (Baseline-after one hour)**	**ΔE2 (Baseline-two weeks)**	**ΔL2 (Baseline-two weeks)**	**ΔE3 (Baseline-four weeks)**	**ΔL3 (Baseline-four weeks)**
SDF	51.76 ± 19.10	-52.76 ± 12.65	68.21 ± 8.07	-62.83 ± 8.47	73.22 ± 9.94	-67.57 ± 10.36
SDF& KI	12.60 ± 14.70	-2.19 ± 5.90	30.31 ± 23.59	-22.73 ± 16.43	28.65 ± 17.39	-27.78 ± 16.94
NSF	11.19 ± 17.14	-4.08 ± 6.09	15.50 ± 16.15	-7.44 ± 5.14	23.23 ± 17.26	-16.45 ± 11.13
***p* value**	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
Post-hoc						
*p*1 value	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
*p*2 value	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
*p*3 value	0.855	0.635	0.064	0.005*	0.434	0.064

Data expressed as mean (SD). p-value was significant (*) if < 0.05. p-value compared between the three agents' groups, p1 value between SDF vs. SDF& KI, p2 value between SDF vs. NSF, and p3 value between NSF vs. SDF& KI.

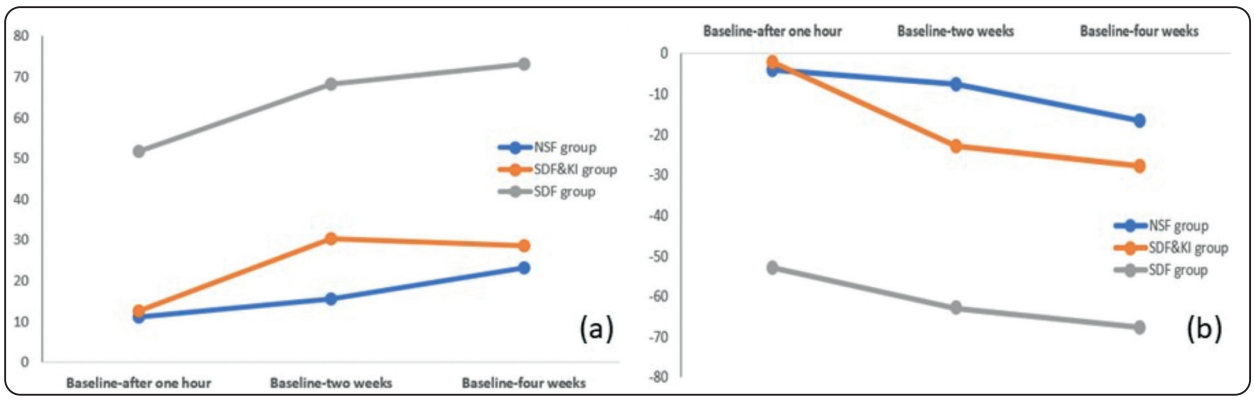

Fig. (2): (a) ΔE and, (b) ΔL enamel staining of the three groups at different time intervals.

DISCUSSION

Recent caries management emphasizes non-restorative, non-invasive, and micro-invasive therapeutic approaches that successfully cease the caries progression at the lesion level and minimize the loss of healthy tooth structure.

SDF represents a promising advancement in caries management. Despite its proven efficacy as a cariostatic agent, the resulting black stains limit its use in the anterior teeth and decrease parent and patient acceptance.

Recently, NSF was introduced as a cariostatic agent with reduced staining potential [9]. Thus, the current study was performed as an in vitro study comparing the staining potential of SDF, SDF+ KI and SNF on demineralized primary teeth enamel.

The current study was designed as a laboratory study since this study type permits the comparison between different interventions under standardized conditions[20]. Primary teeth were selected as cariostatic agents are commonly used to arrest caries in the primary teeth of children with decreased cooperation[23]. Moreover, the effectiveness of cariostatic agents in primary dentition has been confirmed as the enamel is thinner with greater permeability that is about 150 times greater than that in permanent teeth allowing for fluoride diffusion[24].

Several studies have tested the staining potential of cariostatic agents using either bovine teeth[21] or permanent human teeth [3,7,13,22], however, they differ in structure and morphology compared to primary teeth. Also, cariostatic agents were tested on demineralized enamel rather than dentine since cariostatic agents show great success when applied to early carious lesions affecting enamel.

Samples were immersed in a demineralizing solution for seven days to produce simulated demineralized enamel lesions. This complies with Gupte et al. (2021) [13] and El-Desouky et al. (2022) [14] who followed the same demineralization protocol. Then specimens were randomly allocated into three groups with three different treatment approaches [13,14].

The present study investigated the change in color in the three groups at four-time points (before treatment and one hour, two- and four-weeks after treatment) which complies with the time intervals of Nguyen et al. (2017)[25], Espíndola et al. (2020) [7] and Gupte et al. (2021) [13].

Color change was assessed using a digital spectrophotometer as it is amongst the most accurate instruments for color analysis in dentistry. Accurate tooth color measurements can be obtained with a digital spectrophotometer, which is superior to visual analysis, that is subject to observer variability. Additionally, human physiological variables (such as age, emotional state, and fatigue) and lighting conditions can impact the assessment of visual color[27]. In the current study, spectrophotometric assessment was performed according to CIE L*a*b* color analysis to calculate ΔE and ΔL similar to studies assessing tooth color variations either after bleaching or cariostatic agent application in the permanent dentition [7, 13, 25, 26].

In the current study, spectrophotometric results showed that ΔE was increased in the three groups after the cariostatic agents' application at the three-time intervals. Clinically, a color change is visible when ΔE values are higher than 3.7 units [5, 26]. ΔE1, ΔE 2 and ΔE 3 values of the three groups exceeded the critical value of clinical detection (3.7 units).

The current study revealed that SDF exhibited the highest staining potential at the 3 time intervals and the results were found to be statistically significant ($p < 0.001$). Staining was reduced by the application of KI. This goes with the results of Patel et al. (2018) [3] and Gupte et al. (2021) [13] who concluded that SDF can visibly stain dental hard tissues and

that the application of KI following SDF helps in reducing the staining. However, Li et al (2016) [4] reported that SDF+KI-treated carious lesions were stained similarly to applying SDF alone after 30 months. This difference may be attributed to studying the staining effect on root caries in permanent teeth of elderly patients. Also, the results might be affected by the oral environment as well as the extended follow-up period [3, 4, 13].

On the other hand, minimal staining of the enamel of teeth treated by NSF was observed. The staining of the NSF was similar to SDF+KI one hour after application. However, ΔE2, ΔL2, ΔE3, and ΔL3 values indicated less staining effect of NSF on enamel surfaces after 2 and 4 weeks of application, compared to SDF+KI. The results were only statistically significant for ΔL2 (p= 0.005). The results after 2 weeks coincide with the results of the in vitro study of Espíndola et al. (2020) [7] who compared similar cariostatic agents on dentin staining of permanent molars. However, Espíndola et al. (2020) [7] reported an enhancement of the values of ΔE and ΔL after 4 weeks of NSF application. This may be attributed to applying the agents to dentin rather than enamel and the brushing cycles that seemed to remove the stained layer [7].

The limitation of the current study was the assessment of color change under in-vitro conditions. Therefore, further clinical studies are recommended to evaluate the staining effect of NSF on primary teeth under the challenging oral environment over an extended period and to test different concentrations of NSF in vivo to study the cariostatic effect and staining potential on primary teeth.

CONCLUSION

Within the limitations of the current in vitro study, NSF could be a more esthetic alternative to SDF, and SDF+KI when applied to demineralized enamel of primary teeth.

REFERENCES

1. González JIC, Anchundia AKM, Tuarez CAL. Importance of Minimally Invasive Dentistry: A Literature Review. HIV Nursing. 2023;23(3):1823–6-–6.
2. Zameer M, Birajdar SB, Basheer SN, Peeran SW, Peeran SA, Reddy A. Nanosilver fluoride as a caries arresting agent: A narrative. Contemp Pediatr. 2021;2(1):1-13.
3. Patel J, Anthonappa RP, King NM. Evaluation of the staining potential of silver diamine fluoride: in vitro. International journal of paediatric dentistry. 2018;28(5):514-22.
4. Li R, Lo E, Liu B, Wong M, Chu C. Randomized clinical trial on arresting dental root caries through silver diammine fluoride applications in community-dwelling elders. Journal of dentistry. 2016;51:15-20.
5. Zhao IS, Mei ML, Burrow MF, Lo EC-M, Chu C-H. Effect of silver diamine fluoride and potassium iodide treatment on secondary caries prevention and tooth discolouration in cervical glass ionomer cement restoration. International journal of molecular sciences. 2017;18(2):340.
6. Shah S, Bhaskar V, Venkatraghavan K, Choudhary P, Trivedi K. Silver diamine fluoride: a review and current applications. Journal of Advanced Oral Research. 2014; 5(1):25-35.
7. Espíndola-Castro L, Rosenblatt A, Galembeck A, Monteiro G. Dentin staining caused by nano-silver fluoride: a comparative study. Operative dentistry. 2020; 45(4): 435-41.
8. Vijayakumar M, Sabari Lavanya S, Ponnudurai Arangannal JJ, AarthiJ AS. Nano Silver Fluoride-Overview. European Journal of Molecular & Clinical Medicine. 2020;7(2): 6573-80.
9. El-Desouky D, Hanno A, Dowidar K, Hamza SA, El-Desouky LM. Evaluation of the anticariogenic effect of nano silver fluoride on demineralization of enamel in primary teeth (an in vitro study). Alexandria Dental Journal. 2021;46(2):153-9.
10. dos Santos Jr VE, Vasconcelos Filho A, Targino AGR, Flores MAP, Galembeck A, Caldas Jr AF, et al. A new "silver-bullet" to treat caries in children–nano silver fluoride: a randomised clinical trial. Journal of dentistry. 2014;42(8):945-51.
11. Pushpalatha C, Bharkhavy K, Shakir A, Augustine D, Sowmya S, Bahammam HA, et al. The Anticariogenic Efficacy of Nano Silver Fluoride. Frontiers in Bioengineering and Biotechnology. 2022;10:931327.

12. Asghar M, Omar RA, Yahya R, Yap AU, Shaikh MS. Approaches to minimize tooth staining associated with silver diamine fluoride: A systematic review. Journal of Esthetic and Restorative Dentistry. 2023;35(2):322-32.

13. Gupte M, Saraf T, Jawdekar A, Khare S, Tiku A. A comparative analysis of staining propensity of SDF, SDF with potassium iodide and SDF with glutathione biomolecule on demineralized enamel: An in-vitro study. Int J Appl Dent Sci. 2021;7:297-301.

14. El-Desouky DI, Hanno A, Elhamouly Y, Hamza SA, El-Desouky LM, Dowidar KM. Preventive potential of nano silver fluoride versus sodium fluoride varnish on enamel caries like lesions in primary teeth: in vitro study. BMC Oral Health. 2022;22(1):1-10.

15. Chu CH, Mei L, Seneviratne CJ, Lo ECM. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomyces naeslundii biofilms. International journal of paediatric dentistry. 2012;22(1):2-10.

16. Tabatabaian F, Beyabanaki E, Alirezaei P, Epakchi S. Visual and digital tooth shade selection methods, related effective factors and conditions, and their accuracy and precision: A literature review. Journal of Esthetic and Restorative Dentistry. 2021;33(8):1084-104.

17. Knezović D, Zlatarić D, Illeš IŽ, Alajbeg M. In vivo and in vitro evaluations of repeatability and accuracy of VITA Easyshade® Advance 4.0 dental shade-matching device. Acta Stomatologica Croatica. 2015;49(2):112.

18. Javali MA, Abdul Khader M, Alqahtani RM, Almufarrij MJ, Alqahtani TM, Addas MK. Spectrophotometric analysis of dental enamel staining to antiseptic and dietary agents: In vitro study. International Journal of Dentistry. 2020;2020.

19. Parikh AA, Nirupama D, Naveen D, Sindhu J, Nainan MT. Spectrophotometric analysis of crown discoloration induced by various intracanal medicaments: An: in vitro: study. Endodontology. 2021;33(3):149-52.

20. Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials, a review in 3 parts. Part 1: Approval requirements and standardized testing of composite materials according to ISO specifications. Schweizer Monatsschrift fur Zahnmedizin= Revue mensuelle suisse d'odonto-stomatologie= Rivista mensile svizzera di odontologia e stomatologia. 2011;121(9):804-16.

21. Ramlal C, Triana N. Application and Perception of Potassium Iodide Following Silver Diamine Fluoride Treatment. Materials Sciences and Applications. 2022;13(9):506-18.

22. Detsomboonrat P, Thongmak P, Lertpayab P, Aiemsri W, Sooampon S. Optimal concentration of potassium iodide to reduce the black staining of silver diamine fluoride. Journal of dental sciences. 2022;17(1):300-7.

23. Chu C, Lo E. Promoting caries arrest in children with silver diamine fluoride: a review. Oral health & preventive dentistry. 2008;6(4).

24. Teixeira JdA, Melo PCd, Lima MGdS, Mota CCBdO, Lins ECCC, Pereira JRD, et al. Remineralizing potential of nano-silver-fluoride for tooth enamel: an optical coherence tomography analysis. Pesquisa Brasileira em Odontopediatria e Clínica Integrada. 2019;19.

25. Nguyen V, Neill C, Felsenfeld J, Primus C. Potassium iodide. The solution to silver diamine fluoride discoloration. Health. 2017;5(1):555655.

26. Own RM, Wahba NA, Abdelkader SM, El Habashy LM, Eldesouky LM. The effect of enamel pretreatment with nanosilver fluoride on pit and fissure sealant in permanent teeth (in-vitro study). Alexandria Dental Journal. 2022;47(2):189-95.

27. Giudice RL, Pantaleo G, Lizio A, Romeo U, Castiello G, Spagnuolo G, et al. Clinical and spectrophotometric evaluation of LED and laser activated teeth bleaching. The open dentistry journal. 2016;10:242.