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THE ANTIBACTERIAL EFFECT OF NANOSILVER FLUORIDE IN RELATION TO CARIES ACTIVITY IN PRIMARY TEETH: A RANDOMIZED CONTROLLED CLINICAL TRIAL

2021/2022

ABSTRACT

Background: Dental caries is among the most prevalent chronic diseases in children. Minimally invasive dentistry is a highly convenient and efficient method of managing caries in pediatric patients. Recently, Nanosilver fluoride (NSF) has emerged as a promising topical fluoride agent with a potent cariostatic and antibacterial potential. This new anticaries agent is used as an alternative to overcome the drawbacks of topical fluoride and silver diamine fluoride (SDF) in caries prevention.

Objective: To assess the antibacterial effect of NSF in relation to caries activity in dentin caries lesions of primary teeth in comparison to SDF after 1 month.

Methods: Fifty children aged 4 to 6 years old with active dentin caries lesions according to ICDAS score 5 will be recruited from the Pediatric Dentistry clinic at the Faculty of Dentistry, Alexandria University. The children will be equally and randomly allocated into 2 groups; the first group will be treated using NSF, while the second group will be the control group receiving SDF treatment. Microbiological assessment using a microbrush will be done at baseline and follow-up appointments to record the bacterial counts using the suitable culture media, the results will be expressed in colony forming units (CFU). Data regarding the children's oral health will be collected and their dmf index will be scored. The arrest of active carious lesions will be measured after 1 month according to ICDAS criteria and a microbiological assessment will be completed.

Results: The relation between bacterial counts (CFU) and lesion activity for both interventions will be assessed. The data collected will be statistically evaluated with the suitable tests and tabulated.

Keywords: Nanosilver fluoride, silver diamine fluoride, antibacterial, caries arrest, microbiology, primary teeth, pediatric, clinical trial

INTRODUCTION

Dental caries is a chronic disease, ranking among the most highly prevalent diseases in children. According to the Global Burden of Disease study in 2017,[1] untreated dental caries in the deciduous dentition was the 10th most prevalent condition globally, affecting 532 million children worldwide.[2] It is one of the most common infectious diseases of childhood which is evident from the fact that in children, it is 7 times more common than hay fever or allergic rhinitis and 5 times more common than asthma.[3] Under severe conditions, the pain and discomfort associated with dental caries can affect the quality of life of children and may lead to acute and chronic infections, changed eating and sleeping habits, and an increased risk of hospitalization.[4,5]

Among a variety of chemotherapeutic agents that have been tested for preventing and arresting caries. Silver diamine fluoride (SDF) has a proven antibacterial action[6] and releases fluoride ions onto the applied tooth surfaces, therefore, assisting in enamel remineralization[7]. Recently, the promising results of published clinical trials have popularized the use of SDF throughout the world.[8,9] Today, it is available for arresting caries as a simple, non-invasive, low-cost medication.[8,10] The major drawback of SDF is the staining of carious tissue to dark black due to the oxidation of the ionic silver in its formulation, along with reversible slightly painful lesions in oral mucosa caused by accidental contact of the SDF solution with soft tissues, these lesions usually disappear within 48 hours.[11]

New formulations of silver nanoparticles (AgNP) show potent antimicrobial properties against a wide variety of microorganisms.[12–14] Haghgoo et al.[15] suggested the incorporation of nanosilver in varnishes and reported increased antimicrobial properties against cariogenic microorganisms such as *streptococcus mutans* and *streptococcus salivarius*. Nanosilver fluoride (NSF) is an experimental formula obtainable as a yellow solution containing uniformly dispersed silver

nanoparticles and fluoride. It was developed to be an effective anti-caries agent that does not stain the porous dental tissues black as does SDF.[16]

Interestingly, it is unknown if the reduction in caries activity brought about the use of nanosilver fluoride is due to its remineralizing action, antibacterial action, or due to a combination of both.[17,18] There is a need to understand the interaction of topical caries arresting agents like NSF and SDF with the oral microbiome associated with dental caries, since the in vitro findings cannot be extrapolated to the clinical use of NSF.[19]

To our knowledge, no publication has reported the in vivo antimicrobial efficiency of NSF on the primary dentition in comparison to SDF. Therefore, this study will be undertaken to evaluate and compare the clinical antibacterial and cariostatic effectiveness of NSF compared to that of SDF in 4-6 year old children. The null hypothesis of this study is that no statistically significant difference will be detected regarding the antibacterial effect of nanosilver fluoride in relation to caries activity in dentin caries lesions of primary teeth, in comparison to silver diamine fluoride, after one month.

AIM OF THE STUDY

General aim

To assess the antibacterial effect of nanosilver fluoride in relation to caries activity in dentin caries lesions of primary teeth in comparison to silver diamine fluoride after one month.

Specific aims

- To compare the change in *Streptococcus mutans* and *Lactobacilli* counts in dentin caries lesions of primary teeth after one month of the application of nanosilver fluoride or silver diamine fluoride.
- To compare the effect of nanosilver fluoride to that of silver diamine fluoride on caries lesion activity in dentin caries lesions of primary teeth after one month of application according to ICDAS criteria.

MATERIALS AND METHODS

Study Design

This will be a randomized, parallel, two-arm clinical trial, with a 1:1 allocation ratio. It will be set up and reported according to the CONSORT guidelines.[20]

The PICOT question is: will children (4-6 years old) with an active carious lesion (ICDAS code 5) (population; P) using nanosilver fluoride varnish (Intervention; I) in comparison to silver diamine fluoride (comparison; C) show a greater percentage decrease in bacterial counts in the caries lesion (outcome; O) after 1 month follow-up (time; T)?

Study Setting and Location

The study will take place in the Department of Pediatric Dentistry and Dental Public Health at the Faculty of Dentistry and the Department of Medical Microbiology and Immunology at the Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Sample

Sample size estimation

The minimum sample size was calculated based on the results of a previous study.[6] Adopting a power of 80% and a statistical significance level of 5% (α=0.05), 24 children in each group will be needed to generate a statistically significant result. The sample size will be increased to 25 children of both genders per group to compensate for possible drop-out participants. Total sample size= 25x2=50. The sample size estimation was based on Rosner's method[21] and calculated using G*Power 3.1.9.7.[22]

Eligibility criteria

The participants enrolled in this study will be selected after fulfilling the following criteria:

Inclusion Criteria

- Children aged 4-6 years old.
- The presence of at least one active carious lesion on a primary tooth, with a score of 5 according to the International Detection and Assessment System (ICDAS), detected by visual-tactile inspection to assess lesion severity and activity.[23] (Appendix I, Tables 1 and 2)
- Completion of an informed consent to participate in the study.

Exclusion criteria

- Children reporting spontaneous or elicited pain from caries or showing any signs of pulpal infection, swelling, abscess, obvious discoloration of the tooth, or premature mobility.[11]
- Reported usage of local or systemic antibiotics, chlorhexidine or fluoride mouthwashes within the last 2 weeks.[24]
- Children presenting with special health care needs or undergoing medical treatment for chronic or acute diseases affecting salivary flow.
- Allergy or sensitivity to silver or any of the materials included in the study.[11]
- Child weight less than 10 Kg (to avoid concerns for toxicity).[25]

Randomization technique and allocation[26]

Subjects complying with the inclusion criteria will be randomly assigned using a computer-generated list of random numbers to either the NSF or the SDF group (www.random.org). Allocation will be performed by a trial independent individual and the allocation ratio is intended to be equal (1:1).

Allocation concealment[27]

An assistant will be responsible for giving each participant a serial number that will be used for his/her allocation. A duplicate of this number will be kept in an opaque envelope indicating to which group the patient belongs. This envelope will be kept by a trial independent individual who will be assigned the role of opening it only at the time of intervention; so that the group to which the child is allocated is concealed from the investigator.

Grouping

Participants will be randomly and equally allocated to one of the two treatment groups:

- **Test group (NSF):** Twenty-five children meeting the eligibility criteria will receive Nanosilver fluoride.
- **Control group (SDF):** Twenty-five children meeting the eligibility criteria will receive 38% Silver diamine fluoride.

Blinding

The investigator will not be blinded to the treatment type as the SDF solution has a bluish tint and will stain the active caries lesions black, while the NSF solution has a yellowish tint and is not expected to stain the lesions. However, the participants, the statistician, and the microbiologist will be blinded to the treatment group.

Materials

PEG-coated nanosilver fluoride (50 nm average particle size) will be prepared in the Center of Excellence for Research in Regenerative Medicine and its Applications (CERRMA) at the Faculty of Medicine, Alexandria University).

Once all of the above characterization tests and cytotoxicity assays yield satisfactory results, fluoride will then be added. Ten ml of 22,600 ppm of 5% Sodium fluoride varnish will be added to the nanosilver particles in a light proof brown bottle, vigorous stirring will be performed to achieve uniform dispersion of the nanosilver particles.

- 38% silver diamine fluoride: Advantage Arrest, Elevate Oral care (8 mL bottle).*[28]
- Gauze
- Mirror
- Cotton rolls and pelllets
- Tweezers
- Ball ended probe
- Plastic dappen dish
- Petroleum jelly
- Microbrush

Materials for the microbiological assessment

- Microbrush
- Mitis Salivarius Bacitracin (MSB) agar (selective culture media for *S. mutans*), prepared according to the manufacturer's instructions.**
- Rogosa SL agar plates (selective culture media for *lactobacilli*), prepared according to the manufacturer's instructions.***

- Saline as a transport medium.
- Sterile test tubes.
- Sterile plastic Petri dishes.
- Anaerobic gas jar.
- Incubator. ****

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* Elevate Oral Care, LLC, West Palm Beach, FL, USA.
** Difco Laboratories Inc, NJ, USA.
*** Himedia Laboratories, Mumbai, India.
****Red line by binder

Method

First visit: baseline examination

After obtaining the informed consent from the care giver/parent, the researcher will provide oral hygiene instructions to each of the study participants and will inform them about the importance of maintaining good oral hygiene and a proper diet.[29] The patients and caregivers will be informed about the therapeutic intervention to be applied, the possible outcomes, and side effects in detail. All of the examinations and interventions will be done by one calibrated examiner. All of the lesions in the children's oral cavity indicated for treatment with NSF or SDF will be treated and sampled for microbiological analysis.[30]

On the day of the intervention, the children will be asked to refrain from tooth brushing in the morning, as well as eating and drinking (except water) for at least two hours before the appointment. The dmf index will be recorded for each patient.

For caries treatment in all groups, no caries or unsupported enamel will be removed. The patients will be instructed to rinse with a cup of water, then any gross debris will be gently removed with a piece of gauze. The tooth will then be partially isolated using cotton rolls and a saliva ejector. Additionally, petroleum jelly will be applied to the gingiva for added protection. Before applying the intervention, a 2-mm wide microbrush will be rubbed on the carious lesion for 4 seconds to collect the baseline microbiological sample.[6] The microbrush will then be inserted in a sterile test tube containing 1 mL of saline and transported to the microbiology laboratory within 1-2 hours.[31]

Intervention

Test group (NSF):

- 1 drop of NSF will be dispensed into a sterile plastic dappen dish.

- Affected tooth surfaces will be dried with a gentle flow of air.
- The solution will be applied to the carious lesions using a microbrush.
- No more than one drop of NSF will be used for each patient.
- The NSF solution will be left in contact with the tooth surface for 1 minute.[32]
- Any excess will be removed with a cotton pellet to minimize systemic absorption.

Control group (SDF):
It will be applied according to the manufacturer's instructions and the AAPD guidelines for using SDF.[28,30]

- One drop of 38% SDF will be dispensed into a sterile plastic dappen dish.
- Affected tooth surfaces will be dried with a gentle flow of air.
- A microbrush will be dipped into the SDF and dabbed on the side of the dappen dish to remove excess liquid before applying the solution to only the affected tooth surface.
- No more than one drop of SDF will be used for each patient.
- The SDF will be allowed to dry on the lesion for 1 minute.[33]
- Any excess will be removed with a cotton pellet to minimize systemic absorption.

After the application of fluoride agents, all of the children will be instructed not to drink or eat for 1 hour after the appointment, their parents will be asked to observe this instruction.[24] Additionally, for ethical considerations, teeth not treated with SDF or NSF or those showing signs of failure or any new carious lesions that will appear during follow-up period will be appropriately treated.

Microbiological Procedure

1. **Sample dilution**

   All samples will be dispersed by vortexing for 30 seconds then 10-fold serially diluted using sterile saline. A measure of the dilution will then be used for traditional plate culturing methods.[34,35]

2. **Culture**

   Aliquots of 10 ml of each dilution will be inoculated into freshly prepared Mitis Salivarius agar and Rogosa agar media respectively, using a micropipette.

   - Mitis Salivarius agar plates will be incubated anaerobically in an atmosphere containing 10% CO_2 at 37°C for 72 hours to detect *Streptococcus mutans* counts.
   - Rogosa agar plates will be incubated aerobically at 37° C for 48 hours to detect *Lactobacilli* count.

3. **Isolation and Enumeration**

   Following the predetermined incubation period, colonies grown on the specified media will be counted and represented as (CFU/ml). *Streptococcus mutans* will be identified based on their characteristic morphology on Mitis Salivarius agar plates. Similarly, *Lactobacilli* will be identified biochemically and microscopically on the basis of their morphology.

4. **Colony count**

   The number of colonies will be determined and expressed as colony forming units using the following equation.[36]

$$\text{CFU/ml} = \frac{\text{n° of colonies} \times \text{dilution factor}}{\text{Volume taken in ml (1)}}$$

STUDY OUTCOME

Treatment effect will be evaluated after one month by comparing:

- Microbiological assessment of colony forming units (CFU) of *Streptococcus mutans* and *Lactobacilli*.
- Caries activity according to ICDAS criteria.

Follow up

All patients will be recalled after one month. On the day of the recall appointment, patient preparation will be done as mentioned before.

Outcome assessment

- Microbiological assessment will be done as mentioned before and CFU counts will be recorded.
- Lesion activity will be assessed according to the ICDAS criteria. Using a blunt ended probe, if the cavity is found to be shiny and feels hard on gentle probing of the dentin, it will be recorded as an inactive lesion. While if it remains soft or leathery on gentle probing, it will be recorded as an active lesion.

STUDY PLAN

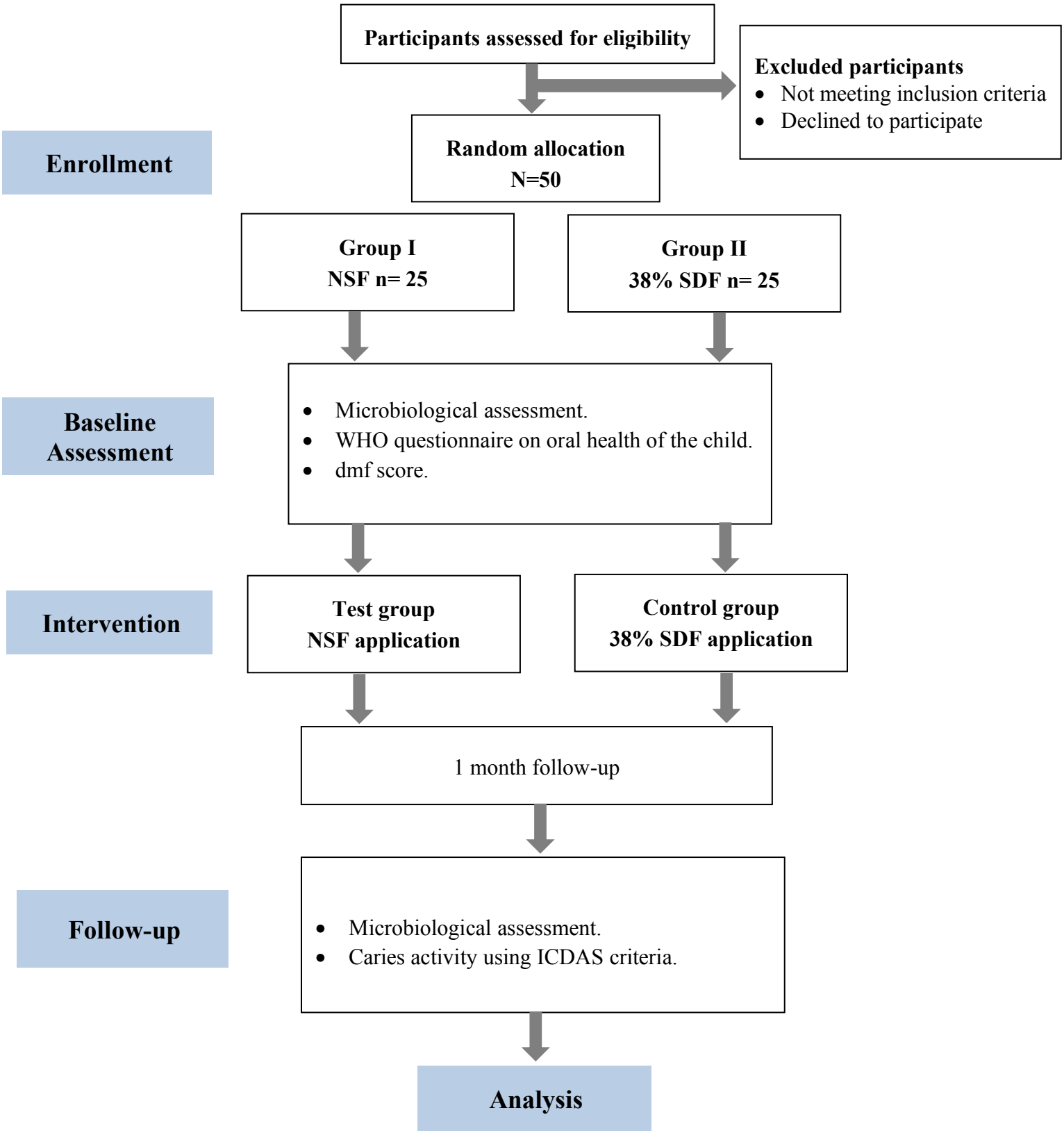

Figure: flow chart of the study plan

STATISTICAL ANALYSIS

The statistical software SPSS version 27.0 for Windows will be used for data analysis. The outcome variable is the number of colony forming units (CFU) of *streptococcus mutans and lactobacilli,* and the number of arrested caries lesions after 1 month in both the SDF and NSF groups. The collected data will be recorded and statistically evaluated.

The Chi square test will be used to compare the difference between the two groups regarding change caries lesion activity, the T-test or the Mann-Whitney U test will be used to analyze the differences between the microbiological samples, Wilcoxon signed rank will be used to assess the changes over time in bacterial counts. Regression analysis will be used to assess the potential confounders such as gender, social status, and dietary habits. The data will be graphically presented with suitable graphs. The level of statistical significance will be set at 5%.

ETHICAL CONSIDERATIONS IN SUBJECTS INVOLVING HUMAN SUBJECTS

1. This research protocol will be approved by the Research Ethics Committee of Alexandria University Faculty of Dentistry (IORG 0008839-IRB No. 0359-12/2021). prior to any research-related activities.

2. All research activities involving human subjects will abide by the Declaration of Helsinki[37] and other ethical guidelines adopted by the Research Ethics Committee of Alexandria University Faculty of Dentistry.

3. Benefits:
   - It will provide a caries arresting treatment for young children with multiple active carious lesions using minimally invasive atraumatic procedures.
   - It will allow overcoming the disadvantages of silver diamine fluoride including black staining of the teeth and any clothes it contacts, soft tissue injury, unpleasant taste for children.
   - Support the use of nanosilver fluoride as a more convenient and more economical substitute to silver diamine fluoride for caries arrest in the primary dentition.

4. Harms/Risks:
   Nothing more than the standard of care and the risk of black discoloration with SDF treatment.

5. Privacy and confidentiality:
   Each participant will be provided with a serial number that will only be accessible to the principal investigator. No data regarding the identity of the participants will be shared under any circumstances. All participants or their guardians must provide a written informed consent prior to any procedures.

DURATION OF THE STUDY

Study Activity	Month								
	1	2	3	4	5	6	7	8	9
Purchase material	✓	✓							
Patient recruitment	✓	✓	✓						
Treatment procedures				✓	✓				
Evaluation Patients will be examined for caries activity and samples will be collected for microbiological assessment					✓	✓			
Data collection and statistical analysis						✓	✓		
Thesis submission								✓	✓

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Appendix I

Table (1): ICDAS severity criteria[38]

Score	Criteria
0	No change in enamel translucency with prolonged air drying
1	First visual change in enamel (after prolonged air drying)
2	Distinct visual change in enamel
3	Localized enamel breakdown or discolored enamel with no visible dentine involvement
4	Underlying dark shadow from dentine
5	Distinct cavity with visible dentine
6	Extensive distinct cavity with visible dentine

Table (2): ICDAS caries activity criteria[38]

ICDAS code	Characteristics of Lesion	
	Active Lesion	**Inactive Lesion**
1, 2 or 3	Surface of enamel is whitish/yellowish opaque with loss of luster; feels rough when the tip of the probe is moved. Lesion is in a plaque stagnation area, i.e.: pits and fissures, near the gingival and approximal surface below the contact point.	Surface of enamel is whitish, brownish or black. Enamel may be shiny and feels hard and smooth when the tip of the probe is moved. For smooth surfaces, caries lesion is typically located at some distance from the gingival margin
4	Probably active	
5 or 6	Cavity feels soft or leathery on gently probing the dentin	Cavity may be shiny and feels hard on gently probing the dentin.