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Research Protocol

Dentistry Section

Remineralising Effects of L-arginine Fluoride Mouthwash versus Sodium Fluoride Mouthwash: A Protocol of an In-vitro Study

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ABSTRACT

Introduction: Preventing dental caries and maintaining oral health are important for children's quality of life, as they profoundly impact overall well being, self-esteem and long-term health outcomes. Despite breakthroughs in preventive dentistry, oral infections and tooth decay remain common among children, highlighting the need for novel and efficient preventive interventions. Although fluoride is a proven preventive measure against tooth decay, L-arginine, a naturally occurring amino acid, has shown promising results in enhancing remineralisation and has gained interest due to its role in maintaining oral health. The combination of L-arginine and fluoride in mouthwash may improve dental health.

Need of the Study: L-arginine fluoride is utilised in oral care products such as varnishes and toothpastes; however, there are no studies examining the efficacy of L-arginine fluoride as a mouthwash.

Aim: To assess the remineralising activity of L-arginine fluoride mouthwash compared to sodium fluoride mouthwash.

Materials and Methods: The present in-vitro study will be conducted in the Department of Paediatric and Preventive Dentistry, Sharad Pawar Dental College, Wardha, Maharashtra, India from September 2024 to December 2025. Forty-two teeth with no white spot lesions or caries will be included, while teeth with lesions, fractures, or wear will be excluded from the study. The teeth will be randomly divided into six groups with different concentrations of L-arginine fluoride, and a control group with sodium fluoride will also be evaluated. The teeth will be assessed for Ca/P ratio using Scanning Electron Microscopy with Energy Dispersive X-ray (SEM-EDX), mineral density using micro-Computed Tomography (micro-CT), and for mineral gain and remineralisation using a specific formula. The data will be statistically analysed using Statistical Package for Social Sciences (SPSS) software version 22.0; a two-way Analysis of Variance (ANOVA) with Bonferroni post-hoc test, a one-way ANOVA with Student-Newman-Keuls (SNK) test, and Kruskal-Wallis one-way ANOVA will be used. A p-value of <0.05 will be considered statistically significant.

Keywords: Biofilm, Caries, Demineralisation, Mineral density, Oral health

INTRODUCTION

Dental caries has a significant worldwide impact, especially on paediatric patients, creating an imperative need for prevention. The development of carious lesions caused by mineral loss occurs during the transition from stable dynamics, characterised by mild or infrequent acidification, to prolonged acidification, which is marked by acidogenic and aciduric phases. To prevent this, proactive techniques, such as antimicrobial agents and fluoride treatment, aim to promote microbial biofilm equilibrium while simultaneously recovering mineral loss through remineralisation [1].

Fluoride administration has had a significant global impact on the decrease of dental caries over the last several decades [2,3]. Enamel remineralisation is promoted by fluoride, which converts hydroxyapatite crystals to fluorapatite, reducing their solubility in the oral environment and consequently decreasing demineralisation [2,3]. However, excessive use of fluoride in mouthwash or other forms has resulted in the development of resistance in acid-producing microbes such as *Streptococcus mutans* (*S. mutans*) [4]. Furthermore, fluoride has limited antibacterial efficacy against cariogenic bacteria. Therefore, techniques that enhance fluoride supplementation are needed to specifically target cariogenic biofilms in individuals at risk of developing caries [5].

Saliva contains amino acids, one of which is L-arginine, a prebiotic. It has gained interest due to its role in maintaining dental health by influencing biofilm ecology. Microorganisms like *S. gordonii* and *S. Sanguis* metabolises arginine (Arg) by breaking it down in saliva or dental plaque via the Arginine Deiminase System (ADS). These bacteria produce an alkaline environment that aids in pH regulation [6]. Because of their alkalogenic characteristics, the presence of these bacteria and the products of their metabolism create an environment that is unsuitable for the survival of cariogenic bacteria [7].

Antimicrobial mouthwashes are commonly used in addition to regular oral hygiene practices to prevent bacterial progression and guard against oral microbial diseases. Commercially available mouthwashes primarily contain ingredients like chlorhexidine, alcohol and sodium fluoride, which have demonstrated antimicrobial properties. However, their potential side-effects, such as staining of teeth or alterations in taste perception, have encouraged the need for newer formulations that are both effective and have fewer side-effects [8,9]. To date, no studies have examined the effect of arginine in fluoride mouthwash on enamel remineralisation. Therefore, the present study aims to assess the remineralising potential of L-arginine fluoride mouthwash compared with sodium fluoride mouthwash. This approach could potentially improve treatment outcomes while maintaining the benefits of fluoride in promoting dental health. The study will be conducted in phases and this is phase 1 of the study.

Aim

The aim of the study is to assess the remineralisation properties of L-arginine fluoride mouthwash and to compare it with sodium fluoride mouthwash.

Objectives

1. To prepare and characterise L-arginine fluoride mouthwash at different concentrations.
2. To determine the remineralisation potential of L-arginine fluoride mouthwash.
3. To compare the remineralising activities of L-arginine fluoride and sodium fluoride mouthwash.

Alternate hypothesis: There is a significant difference in the remineralising effects between L-arginine fluoride and sodium fluoride mouthwash.

Null hypothesis: There is no significant difference in the remineralisation properties of L-arginine fluoride when compared with sodium fluoride mouthwash.

REVIEW OF LITERATURE

The effectiveness of arginine in reducing caries is well acknowledged. It has been used in supplements, toothpaste, varnish and mouthwash. To further enhance its activity, the present study plans to combine it with fluoride. Porksen CJ et al., evaluated the decrease in relative risk in child patients in comparison to the daily use of arginine-containing lozenges and two strains of probiotics. A 2% arginine lozenge was given to the intervention group (n=141), while the placebo group received a placebo lozenge. Each group was provided with a toothpaste containing 1,450 ppm fluoride. At baseline and follow-up, the first permanent molar, canine and second molar were clinically and radiographically assessed. Examination of the relative risk revealed that the intervention group had fewer active caries lesions (15.3%), greater regression (0.3%), and less caries advancement (13.6%), thereby demonstrating that fluoride combined with arginine is the most effective treatment [10].

In a study, Goyal V et al., assessed the effectiveness of arginine as a caries-prevention agent. It has been proven to work synergistically with fluoride to reduce caries. Arginine was shown to be useful in reducing cavities at a concentration of 1.5% among children and adults, and adding it to fluoride pastes can be extremely beneficial in tackling the increase in dental caries. It not only changes the oral biofilm but also speeds up the absorption of fluoride and strengthens the remineralisation process [11].

Kuriki N et al., conducted a study to investigate the potential of arginine preparations for biofilm reduction. For the study, in-situ biofilm models were used to assess the biofilm. The study concluded that oral flora diversity was altered by the use of an 8% arginine solution, along with an increase in the concentration of ammonium ions, suggesting the effectiveness of arginine as a prebiotic in improving oral health [12].

Bijle MN et al., aimed to analyse the mineral uptake capacity of Arginine (Arg) in Sodium Fluoride (NaF) toothpaste. An experimental group of 50 participants (n=10) was divided into five groups and exposed to artificial lesion formation. A pH cycling of up to 10 days was used to treat specimens in the following groups: (1-5) 2% Arg-NaF, 4% Arg-NaF, 8% Arg-NaF, NaF, and deionised water. Inductively Coupled Plasma-Emission Optical Spectrometry (ICP-EOS) and Fourier Transform Infrared Spectroscopy (FTIR) were used to analyse the pH, fluoride, and Sodium Chloride (NaCl) components in the test solutions. The specimens' mineral density was assessed using micro-CT, the Ca/P ratio and surface fluorine concentration were determined using Energy Dispersive X-ray Spectroscopy (EDS), and Enamel Fluoride Uptake (EFU) was quantified using the acid-etch technique. This indicated that adding 2% arginine to NaF toothpaste greatly enhanced the remineralisation of enamel caries-like lesions compared to NaF toothpaste [13].

Bijle MN et al., also aimed to investigate the remineralisation potential and HGF-1 cytotoxicity of Arg in 5% NaF varnish. The varnish was made utilising two distinct forms of L-arginine, namely L-arginine monohydrochloride and L-arginine at 2%, 4% and 8% w/v, with 5% NaF varnish as a control. Enamel specimens with no enamel defects and varnish treatment were demineralised for four days, and surface roughness was measured using an Atomic Force Microscopy (AFM) (n=3). Enamel specimens with artificial caries development were then subjected to a remineralisation cycle (n=6) and an 8-day pH cycle (n=3). The remineralisation assay measured enamel fluoride absorption and Ca/P ratio content. During the pH cycling, EDX mapping for Ca/F content and mineral density using micro-CT were evaluated [9].

MATERIALS AND METHODS

The present in-vitro investigation will be conducted in the Department of Paediatric and Preventive Dentistry, Sharad Pawar Dental College in Wardha, Maharashtra, India, from September 2024 to December 2025. The Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research granted ethical permission for the study (DMIHER(DU)/IEC/2024/45).

Sample size calculation: The sample size was determined using the formula for the absolute difference between two means, based on a prior study conducted by Bijle MN et al., which followed a similar procedure [13].

$N=\sigma^2 (z1\text{-}\beta+z1\text{-}\alpha/2)^2(\mu0\text{-}\mu1)^2$

$N=0.42\ (0.84N=\sigma^2(z1\text{-}\beta+z1\text{-}\alpha/2)^2(\mu0\text{-}\mu1)$

$2N=0.42\ (0.84+1.96)^2(1.57\text{-}2)^2$

Total N=42

$\mu0$=population mean

$\mu1$=mean of study population

N=sample size of study population

σ=variance of study population (0.648)

α=probability of type I error (usually 0.05)

β=probability of type II error (usually 0.2)

z=critical Z value for a given α

Forty-two samples will be included in the study. They will be randomly divided into six groups: Group 1- 2% Arg-F, Group 2- 4% Arg-F, Group 3- 6% Arg-F, Group 4- 8% Arg-F, Group 5- 10% Arg-F, and Group 6- 2% NaF, which will act as the control group. Each group will consist of seven samples.

Inclusion criteria: Extracted permanent teeth that are free of white spot lesions or carious lesions will be included in the study.

Exclusion criteria: Teeth with any carious lesions or those exhibiting white spots, visible fracture lines, or any signs of wear or enamel defects, such as hypomineralised enamel, enamel hypoplasia, or fluorosis, will be excluded from the study.

Study Procedure

There is no existing study that has prepared the arginine fluoride mouthwash; hence, in the present study, it shall be prepared according to the method described by Bijle MN et al., [13]. Commercially available L-arginine powder (PCT0302-25G) will be mixed with commercially available Sodium Fluoride mouthwash (Listerine, containing sodium fluoride 0.02%, water, sorbitol, sodium lauryl sulphate, eucalyptol, flavor, methyl salicylate, thymol, phosphoric acid, sucralose, menthol, disodium phosphate and alcohol 21.6%) in a sterile container at concentrations of 2%, 4%, 6%, 8% and 10.

Preparation of enamel specimen: Extracted permanent teeth without any enamel defects, such as white spot lesions, hypomineralised enamel, enamel hypoplasia, or fluorosis, will be considered for the study. Forty-two enamel specimens will be produced using the procedure outlined by Bijle MN et al., and will be stored at 4°C in deionised water until use. They will be divided equally among six groups, with seven samples in each

group [13]. The experimental groups (groups 1 to 5) will consist of 2%, 4%, 6%, 8% and 10% L-arginine fluoride, respectively, with NaF serving as the control (sodium fluoride 0.02%, water, sorbitol, sodium lauryl sulphate, eucalyptus, flavor, methyl salicylate, thymol, phosphoric acid, sucralose, menthol, disodium phosphate and alcohol 21.6%). The specimens will be mounted horizontally in self-cured acrylic resin in individual plastic moulds. The specimens will be covered during the moulding period to avoid dehydration.

Artificial incipient caries-like lesion formation: As stated in a previous study by Bijle MN et al., one-third of the enamel surfaces of the 42 specimens will be coated with nail varnish to create a control region [13]. The demineralisation solution will be prepared using analytical-grade chemicals. Artificial caries will be created on the remaining portion, which will then be immersed in the demineralisation solution. After demineralising the enamel specimens, they will be thoroughly rinsed with deionised water to remove any remaining demineralising solution. Subsequently, nail varnish will be applied to cover the middle one-third of the enamel surface, ensuring this area is protected and serves as a demineralised control region. This varnished middle section will remain untreated in future procedures, allowing for comparison. The last one-third will be reserved for future procedures. This method facilitates the assessment of treatment effects on enamel remineralisation by providing a controlled, untreated region for comparison [14].

Mouthwash treatment: Before immersing the teeth in mouthwash, artificial saliva will be produced in the manner stated by Bijle MN et al., [13]. The teeth will be immersed in 20 mL of their assigned mouthwash for 30 seconds, respectively. The teeth will then be immersed in artificial saliva at 37°C for six hours.

pH-Cycling model: Each day of the eight-day cycle, the blocks containing the implanted enamel samples will be submerged at 37°C for two hours of demineralisation and 22 hours of remineralisation. The proportions of demineralising and remineralising solutions will be selected based on the enamel surface area. The demineralising and remineralising solutions will be replaced every fourth day with a new solution. The enamel remineralisation will be assessed at the end of the eight-day cycle [14].

The enamel specimens will then be analysed for the Ca/P ratio using SEM-EDX and for mineral density using micro-CT at T0 (before the procedure), T1 (after demineralisation on day 4), and T2 (at the end of the pH-cycling). The percent remineralisation and mineral gain will be calculated using the following equations:

Mineral Gain=$\Delta Zd-\Delta Zr$

% Remineralisation=$(\Delta Zd-\Delta Zr/\Delta Zd)\times 100$

(ΔZd= MD difference between T0 and T1, ΔZr=MD difference between T2 and T0) [9,13]

Outcome

Primary outcome: The L-arginine fluoride mouthwash will show an increase in remineralising potential with an increase in the concentration of L-arginine.

Secondary outcome: The L-arginine fluoride mouthwash will show better results compared to the sodium fluoride mouthwash.

STATISTICAL ANALYSIS

The data will be subjected to statistical analysis using the SPSS (IBM SPSS, Chicago, IL, USA), software version 22.0. A two-way ANOVA and the Bonferroni post-hoc test will be used to assess the results for estimated Ca/P and mineral density at all the given time periods: T0, T1 and T2. Mineral gain and percentage remineralisation will be computed using a one-way ANOVA with the SNK test.

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