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Comparative Evaluation Of Pediatric Dentifrices In Remineralizing Simulated Carious Lesions On Enamel: An In Vitro Approach

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ABSTRACT

Purpose: This study aimed to assess the remineralizing efficacy of three widely available pediatric dentifrices on artificially created carious lesions in primary teeth using high-resolution SEM-EDXA.

Materials and methods: 30 decay-free primary teeth were demineralized for 96 hours and then cut into 30 sections longitudinally. The sections were randomly designated to three groups, each comprising 10 samples. Group A- Fluoridated dentifrice (KidsBunny), Group B- Non- Non-fluoridated dentifrice (MeeMee), Group C- Herbal dentifrice (MamaEarth). The samples were exposed to a 7-day pH cycle for remineralization and were then analyzed using SEM-EDXA.

Results: The fluoridated dentifrice group showed a statistically significant increase in remineralizing potential. There was a varied difference in the remineralizing potential across all three groups. The herbal dentifrice group showed traces of added elements such as Iodine, Barium, and Silicon after remineralization.

Conclusion: Fluoridated dentifrice (KidsBunny) showed increased remineralizing potential. Herbal dentrifice consisting of various extra elements that can prevent the tooth from acid erosion, oral infections, and aid in strengthening the enamel. Non-fluoridated dentrifice did not show remineralisation potential or elements being added post-remineralisation. Hence, different types of dentifrices can be prescribed to patients based on an individual's food habits, diet pattern, and caries risk..

Keywords: *Remineralization, SEM-EDXA, Ca/P ratio, Fluoridated toothpaste, dentrifices .*

1. INTRODUCTION

Among the most prevalent and longstanding oral health issues, dental caries continues to be a major concern for dental professionals. Although its prevalence is significantly reduced in developed countries, despite implementing several preventive strategies, such as fluoridation protocols, pit and fissure sealant application, sugar alternatives, anticipatory guidance, and dental caries vaccine, its occurrence in primary dentition in developing nations has remained relatively unchanged.[1,2] Increased sugar consumption and decreased exposure to fluoride pose a significant risk to the development of dental decay in the anterior teeth of children.[3] The process of dental caries formation occurs during the alternating phases of mineral loss (demineralization) and mineral recovery (remineralization) of the enamel and dentin. When calcium (Ca) and phosphate (P) ions are externally delivered into the weakened enamel structure, leading to a net restoration of mineral content, it results in remineralization.[4] Whereas demineralization starts at the microscopic level, affecting the crystalline matrix of the enamel or dentin, and if not terminated, it can continue progressively, resulting in cavitation.[5]

The dynamic balance between the pathogenic and protective variables is vital to necessitate the advancement or reversal of the carious process. Fluoride, plays a key role as a protective agent against dental caries and has been historically employed to remineralize and prevent the formation of new carious lesions.[6] The importance of early identification and non-invasive methods to facilitate the remineralization of carious lesions has led to the innovation of newer materials, one such being the casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) nanocomplexes.[7] With the developing trend in the usage of organic and naturally sourced ingredients [8,9], the incorporation of herbal compounds in dentifrices is of profound interest in the field of caries prevention.[10]

As there is a paucity of literature comparing the remineralizing efficacy of fluoridated toothpaste with non-fluoridated toothpaste and herbal toothpaste, this study was carried out to investigate the concentration of calcium and phosphate on artificial caries lesion in primary teeth after treatment with dentifrices incorporated with Tri-calcium phosphate (TCP) and with Fluoride (500 ppm) and dentifrice with natural substance like stevia, xylitol. The values were measured under Hi-Resolution SEM with energy dispersive X-ray analysis. (HR SEM-EDXA)

2. MATERIALS AND METHODS:

This in vitro study was carried out using non-carious teeth, which were extracted or exfoliated and sourced from various hospitals and clinics. The sample size was calculated using G-Power software, using the following parameters: Effect size, $f = 0.25$, α err prob = 0.05, Power ($1-\beta$ err prob) = 0.91. According to these calculations, a sample size of approximately 30 was determined.

A total of 30 non-carious teeth stored in sterile distilled water were used. The samples included were extracted caries-free primary anterior teeth and teeth extracted due to pre-shedding mobility. The samples excluded were permanent teeth, carious, hypoplastic, and discolored teeth which had white spots and cracked areas. This invitro study was authorised by the Institutional Scientific Committee, under IRB number SRMIEC-STO521-744.

3. DEMINERALIZATION PROCESS:

Initially, the tooth samples were removed from sterile distilled water and dried completely. A nail varnish coat was applied to all samples after drying to resist the action of acid, except for the region containing a rectangular window 2 cm × 1 cm wide on the tooth surface for demineralization. The specimens were dipped in a demineralizing solution that had a pH of 4.5 for 96 hours for the demineralized lesions to develop. The demineralizing solution was formulated by mixing 2.2 mM potassium hydrogen orthophosphate, 2.2 mM calcium chloride, and an unmixed solution of 0.05 M acetic acid. To achieve the desirable pH 4.5, 1 M potassium hydroxide was added to this. For preparation, 1050 ml of distilled water was measured into a beaker to which 2.2 g of calcium chloride, 56 g of potassium hydroxide, 3 g of acetic acid and 2.2 g of potassium hydrogen orthophosphate was added. [11,12]

The samples were then sectioned longitudinally in labio-lingual direction with a diamond disc, in such a way that each section contains a part of normal and demineralized enamel. After demineralization process, a total of 30 sound tooth sections were obtained with 10 sections in each group. Following randomization, the samples were divided into three groups, with each group assigned a commercially available toothpaste. Group A (Kids Bunny) – 10 samples, Group B (Mee Mee) – 10 samples, Group C (Mamaearth) - 10 samples. (Fig 1) The 30 samples after demineralization were studied under SEM-EDXA to measure the mineral content of each tooth. The obtained values were documented and systematically tabulated.

4. REMINERALIZATION PROCESS:

The preparation of remineralizing solution was done incorporating 0.5 mM calcium chloride, 0.9 mM of sodium dihydrogen phosphate and 0.15 M of potassium chloride with a pH of 7. After which, 0.1665g of calcium chloride, 0.108g of sodium dihydrogen phosphate, and 11.25g of potassium chloride were sequentially added to 152 ml of distilled water.[11,12] The dentifrice solution was formulated by mixing dentifrice with deionized water in a ratio of 3:1. It was prepared by the stirring method with the help of stirring rod to get it homogeneously mixed. Prior to each pH cycle, a fresh solution for each dentifrice was made and stored throughout the study. In the pH cycle, the specimens were exposed to various solutions for a set time. Conferring to Duijsters and Ten Cate, the pH cycle employed was

Phase 1: Application of supernatant dentrifice solution (5 ml per sample) for 60 seconds.

Phase 2: Immersion in demineralizing suspension (10 ml per sample) for 3 hours.

Phase 3: Immersion in remineralizing suspension(10 ml per sample) for 2 hours.

Phase 4: Reapplication of supernatant dentrifice solution (5 ml per sample) for 60 seconds.

Phase 5: Exposure to demineralizing solution (10 ml per sample) for an additional 3 hours.

Phase 6: A third treatment with supernatant dentrifice solution (5 ml per sample) for 60 seconds.

Phase 7: Overnight immersion in remineralizing suspension (10 ml per tooth).

Phase 8: Continue for 7 days.

On completion of the 7 day pH cycle, the mineral content of the remineralized sections was assessed by SEM-EDXA. (Figures 2-5). Both the demineralized and remineralized samples had their mineral content quantified, tabulated, and statistically analysed.

SPSS VERSION 25.0 was used for the statistical analysis. The Wilcoxon signed rank was done to compare the demineralization and remineralization of Ca/P ratio in group I and II. The Paired t-test was employed to examine the Calcium-Phosphate ratio between the demineralization and remineralization in group III. The Kruskal Wallis test was used to compare the remineralization of C/P ratio between the three groups. The statistical significance was kept at p – value less than or equal to 0.05.

5. RESULTS:

The present study evaluated the remineralization efficacy of three pediatric dentifrices on artificial enamel subsurface lesions using SEM-EDXA. The different elements that were present in the remineralised portion of the tooth are given in Table 1. Energy-dispersive X-ray spectroscopy (EDX) was used to quantify the weight percentage of calcium and phosphorus in both demineralized and remineralized enamel samples within each experimental group (Figs. 6-8). The Ca/P ratios were obtained from each group (Table 2).

To analyse the data statistically, the Wilcoxon signed-rank test, paired t-test, and Kruskal-Wallis test were utilized. The Wilcoxon signed ranked test was used to compare the Ca/P ratio of demineralized and remineralized enamel samples between Group A and Group C. A significant difference in the Ca/P ratio between demineralized and remineralized samples was observed in Group A [p- value:0.022]. The Paired t-test was used to compare the Ca/P ratio of demineralization and remineralization enamel samples between Group A and Group B. The Kruskal-Wallis test compared the means of remineralization of Ca/P ratio between the three groups (Table 3). The Ca/P ratio did not differ statistically between the groups.

6. DISCUSSION:

During phases of neutral pH, remineralization occurs when minerals are redeposited into enamel lesions, and it can also occur concurrently with the progression of carious lesions.[13] Over the past two decades, a novel in vitro method known as pH-cycling has been developed to evaluate the efficacy of caries-preventive treatments. This technique involves subjecting dental specimens to alternating cycles of demineralizing and remineralizing solutions daily. Research using this model has demonstrated that fluoride initially promotes enamel remineralization; however, prolonged exposure may inhibit both demineralization and remineralization processes.[14] The concentration and pH of the solutions were maintained within the physiological range of oral fluids. To prevent saturation and ensure experimental consistency, remineralizing and demineralizing solutions were made fresh on a daily basis prior to each pH cycle, with pH levels verified before use. In the literature, two modified pH-cycling protocols have been proposed for studies on primary teeth: one lasting 7 days, and another extended to 10 days with 0.25 ppm fluoride incorporated into both solutions. The 7-day pH-cycling model was employed in this study. However, the reduced duration may limit the ability of the model to accurately replicate the natural dynamics of enamel demineralization and remineralization.[12]

The remineralizing efficacy is evaluated by measuring the mineral content in the samples with the help of SEM-EDXA. The Ca/P ratio after remineralization increased when compared to demineralization. The maximum Ca/P ratio seen in Group A, B, C are 3.75, 2.57, 3.36 respectively (Table4). The mean and standard deviation of the samples formed after demineralization showed no statistically significant difference from each other. This shows that, despite the fact that the specimens were sectioned from different teeth, variances between them had no effect on the demineralization process.

From the results obtained, it can be seen that the remineralizing efficacy of Kids Bunny toothpaste showed statistically significant variation in the Ca/P ratio between the demineralized and remineralized enamel. This can be attributed to the fluoride concentration (500 ppm) present in the dentrifice. This is in line with the findings of the research done by Gujarathi, et al 2015, which showed that fluoride-based tooth paste showed more remineralizing potential than non-fluoridated tooth pastes.[12] The remineralizing potential of all three groups seems to have minor differences compared to each other. But it is seen that there was no statistical significance difference when the three groups were compared. This is in contradiction to the study done by Ranjana et al in 2021, which stated that non-fluoridated dentrifices showed more remineralizing potential than the herbal-based dentrifices.[15]

The SEM EDX results showed various elements present in the demineralized and remineralized samples. Table 1 illustrates the various elements present in Groups A, B, and C after remineralization. Calcium and phosphate ions are widely recognized for their essential role in promoting enamel remineralization. Additionally, emerging evidence suggests that silicon may contribute to enhancing the acid resistance of enamel crystals.[16] For children suffering from gastroesophageal reflux disease, it is advisable to modify their dietary habits and these children can benefit by using toothpaste containing Silicon, which will prevent the teeth from erosion and decay.

It is noted that several elements were seen in the samples after remineralization. Noticeably, in Group C (Mama Earth) various new elements such as Iodine, Barium and Sulphur were detected in trace amounts, which were not present in the other two groups. A study done by Abdel Naser Zaid in 2011, concluded that Iodine prevents oral and throat infections and sulphur has an anti-inflammatory and astringent action, which helps to speed up the restoration and regeneration of the oral mucosa lining.[17] So, herbal dentrifices can be used in children. It also indicates that barium levels may serve as biomarkers to evaluate the appropriate timing for initiating the weaning process in infants.[18]

One limitation of this study lies in the challenge of accurately replicating the natural composition and flow rate of human saliva under laboratory conditions. Additionally, the artificial carious lesions developed over a few days in vitro, whereas comparable lesion development in a clinical setting typically occurs over approximately 12 months.[12] Moreover, in vitro studies do not fully replicate the natural oral environment due to the absence of enzymatic systems needed for the hydrolysis of sodium monofluorophosphate. These models also fail to reproduce the antiplaque activity of amines, as well as the dynamic variations in both the quantity and composition of saliva that occur in the mouth.[19] The durations of demineralization and remineralization cycles in vitro are significantly shorter than those observed under in vivo conditions. Accurately replicating the oral environment remains challenging, particularly due to the frequent pH fluctuations that occur in response to individual dietary habits and oral hygiene practices.[20]

7. CONCLUSION:

From the observations from this present study, it can be concluded that, the fluoridated toothpaste, Kids Bunny group, showed increased remineralizing potential. The non-fluoridated, Mee Mee toothpaste group did not show any reminerlization potential. However, there is a presence of certain trace amount elements such as silicon added in samples of herbal toothpaste, Mama Earth group after remineralisation, which potentially can prevent the tooth from erosion due to acid and aids in strengthening the enamel.

Hence, different types of dentifrices can be prescribed to the patient based on the individual’s food habits, diet pattern, and caries risk.

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CONFLICTS OF INTEREST:

There are no conflicts of interest.

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