Comparative Evaluation of Root Caries Remineralization Effect of Plain Milk, 5 ppm of Fluoridated Milk, and 5 ppm of Sodium Fluoride in Deionized Water Using Surface Microhardness Test: An In Vitro Study

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Background: There is an increase in longevity of teeth retained in elderly population, leading to increased risk of root caries. Therefore, new and affordable preventive strategies are in need to reduce this problem. Hence, the aim and objective of the study was to assess and compare the root caries remineralization effect of plain milk, 5 ppm of fluoridated milk, and 5 ppm of NaF in deionized water. Materials and Methods: The study was an in vitro experimental design. Sixty root samples were divided into five groups (sound root, demineralized root treated in deionized water, plain milk, 5-ppm fluoridated milk, and 5-ppm fluoridated deionized water) of 12 samples each after baseline surface microhardness analysis and standardization was carried out in the study. All groups except the sound root were subjected to demineralization procedure for 4 days at 37°C. The demineralized four groups were treated to test solutions and subjected to pH cycling for 14 days. This was followed with assessment of postintervention surface microhardness. Student’s paired t test was used for comparing surface within groups. One-way analysis of variance test and post hoc Tukey’s test were conducted for between groups’ comparison. Results: There was a significant difference between groups (P < 0.05). The results of post hoc showed that significant difference was found between the group sound root and demineralized treated with 5-ppm fluoridated milk and 5-ppm fluoridated deionized water with an effect size of 2.15 and 2.87, with CI (–26.8 to –8.1) and (–36 to –17.3), respectively. There was a significant difference in all the groups when compared within the group (P < 0.05). Conclusion: Plain milk, 5-ppm fluoridated milk, and 5-ppm fluoridated deionized water showed remineralizing potential against demineralized in vitro root caries samples. 5-ppm fluoridated deionized water and 5-ppm fluoridated milk have a better effect compared to plain milk.

Keywords: 5-ppm fluoridated milk, pH cycling, root caries, Vickers microhardness test

INTRODUCTION

Dental caries is a multifactorial disease. The prevalence and incidence of dental caries in a population is influenced by various risk factors such as sex, age, socioeconomic status, dietary patterns, and oral hygiene habits.⁴ Dental caries affects all age group

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and it is known to be one of the most significant health problems faced by older adults.[3] More than half of the elderly who are affected with either coronal or root caries (RC) are dentate. There is an increase in dental caries as the age advances to quinquagenarian (50s), sexagenarian (60s), septuagenarian (70s), octogenarian (80s), and nonagenarians (90s). Dental caries development is due to the increasing longevity of the elderly population and the increase in tooth retention in these age groups. As there is an increase in longevity and more the number of teeth, there is an increased risk of RC.[3]

With changing demographics in developed and developing countries, the advent is more in effective preventive oral health treatment programs rather than curative programs. Several studies of RC indicate that it is becoming an influential problem in aging adults due to limited access to preventive care and also restorative treatment.[4,5]

Therefore, new preventive approaches are necessary to reduce this issue. A number of studies have shown the effectiveness of topical fluoride application, such as dentifrices, fluoride solution, paint form, gels, varnishes, and mouth rinses. These chemical agents reported across the literature were enormous and no single intervention can be identified as a “gold standard” for the remineralization and prevention of carious lesions on root surfaces.[6] The value of alternative fluoride applications, such as milk fluoridation, is still being discussed and only a few number of studies are available on the preventive effect related to RC. This concept is also supported by the posteruptive fluoride effect in vitro and in situ investigations showing that the mechanism of action of fluoride can be attributed mainly on its influence on demineralization and remineralization kinetics of dental hard tissues.[7]

Milk is an easily accessible material and could be an ideal vehicle for fluoride administration. Milk is also rich in carbohydrate, fat, protein, biological enzymes, vitamins, and minerals such as calcium.[8] Milk is a readily available oral lubricant and can be used for xerostomia, which not only provides lubrication to the oral tissues but may also aid in buffering of acids and cause remineralization of dental hard tissues; this is due to the presence of calcium and phosphate.[9] Milk is a food with high nutritional quality and its moisturizing properties would certainly benefit patients with xerostomia. Therefore, such patients may find milk of value as a saliva substitute to reduce the oral health problems caused due to hyposalivation.[10] Considering the cost-effectiveness, accessibility, and availability of milk, it can be used as a vehicle to deliver fluoride for the prevention of RC.

To date, only few researches[8,11-13] have addressed the effectiveness of fluoridated milk in RC prevention. Hence, the aim and objective of this study was to assess and compare the RC remineralization effect of plain milk, 5 ppm of fluoridated milk, and 5 ppm of sodium fluoride in deionized water using surface microhardness test.

**Materials and Methods**

This study is an experimental, randomized control trial, in vitro parallel group study design. Ethical clearance was obtained from Institutional Review Board, Bapuji Dental College and Hospital, Davangere. Sample size was calculated using G*Power software, version 3.0.10 Heinrich-Heine-Universität, Düsseldorf, Germany with a test family of F tests with α = 0.05, power of the study (1 – β) = 0.8, and effect size = 0.4. The estimated sample size for five groups was 55 rounded off to 60 with each group consisting of 12 samples. Freshly extracted human upper and lower premolar teeth (first and second premolar teeth) for orthodontic reason were selected in this study. Teeth with signs of RC, cracks, fracture, resorption of root, and teeth extracted due to periodontal disease (recession) were excluded from the study. Informed consent was obtained from the patients who underwent extraction of premolars. Samples were obtained 3 month prior to the start of the study. The teeth were cleansed of visible blood and gross debris and maintained in a hydrated state in 0.9% sodium chloride (NaCl) and 0.1% thymol in a container that was sealed and labeled.[9] Teeth were sectioned 1 mm above the cementoenamel junction (CEJ) with a slow-speed diamond disc (NSK micro-motor handpiece). Crowns were discarded and the roots were used for the study. The roots were sectioned mesiodistally into lingual and buccal halves using a diamond disc bur (NSK micro-motor handpiece) under cool water spray. Following sample preparation, a 4 mm × 4 mm working window was marked on the buccal and lingual surfaces of all the samples at the coronal 1/3 of the root to establish more working area. The area of the root other than the working window was covered with nail varnish (Colorama Nail Varnish, Maybelline, Manhattan, New York, USA) making it resistant to acid attack. The root samples were mounted on acrylic cube with size of 1 × 1 inch for surface hardness testing. The samples were numbered and labeled; a total of 74 samples were carried out considering anticipatory breakage and standardization. The mounted root samples were subjected to polishing with acrylic trimming burs and also with abrasive sand papers (silicon carbide papers).
200-, 400-, 600-, 800-, and 1200-grit, respectively, for surface hardness testing[14] [Figure 1].

The baseline surface microhardness of the specimens was determined using digital microhardness tester (Reichert, Vienna, Wien, Austria, Sr. No. 3637) with a Vickers elongated diamond pyramid indenter and an ×40 objective lens. A load of 50 g was applied to the surface in the 4 × 4 working window for 10 s.[15]

A total of 74 samples were assessed for the baseline hardness test. Fourteen samples were excluded from the study due to extreme values. The randomization was carried out by a person not involved in the study using random number table.[16] Selected 60 samples within the standardized hardness range were randomly allocated to five groups with 12 samples in each group [Figure 2]. The root samples were immersed in 200 mL of caries inducing solution (2.2-mM CaCl$_2$·2H$_2$O, 2.2-mM KH$_2$PO$_4$, and 50-mM acetate) for 4 days (96 h) with 4.6 pH and maintained at a constant temperature of 37°C, in an incubator to obtain artificial caries formation and simulating an effective area of demineralization.[17]

One milligram of NaF was mixed with 200 mL of milk and stirred with a stirring rod to get 5 ppm of fluoridated milk. pH cycling model was carried out to simulate the process of demineralization and remineralization that occurs in the oral cavity in a dynamic way. Each cycle was carried out with demineralization solution for 4 h, treatment solution for 6 h, and 14 h in the remineralization solution; all samples of Groups 2–5 were pH cycled for 2 weeks. Before changing to another solution, the samples were thoroughly rinsed with deionized water. 250 mL of demineralization and remineralization solutions were freshly prepared every third day (every 72 h) and the treatment solutions were prepared every day and used with continuous stirring throughout the experiment. This process continued for 14 days[18] [Figure 3].

Post intervention surface microhardness (PISMH) was assessed after 14 days using Vickers microhardness tester. Data obtained were compiled in Microsoft Excel, Redmond, Washington, United states of America spread sheet. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), IBM, Chicago, Illinois software program, version 20.0. Nature of the data was checked using Kolmogorov–Smirnov test and Shapiro–Wilk test. It was found that data were normally distributed, and hence parametric tests were applied. One-way analysis of variance was applied for multiple group comparison followed by post hoc Tukey’s test to know the difference between the groups. Student’s paired $t$ test was used for comparing surface microhardness at baseline and postintervention within each group.

**RESULTS**

No significant difference was observed between the five interventional groups at baseline, with an $F$ value of 1.79
and $P$ value of 0.144. The mean and standard deviation at baseline for Groups 1–5 were 52.34 ± 8.14, 52.75 ± 6.57, 52.78 ± 8.55, 59.72 ± 10.16, and 56.37 ± 7.54, respectively. The results showed that there was statistically significant difference between the intervention groups with $F$ value of 31.93 and $P$ value of 0.0001 between the groups with a gradual increase in mean value [Graph 1]. Post hoc analysis between the interventional groups showed that a significant difference was found between all the groups except Groups 1 and 2 with a $P$ value of 0.323, Groups 1 and 3 with a $P$ value of 0.171, and Groups 4 and 5 with $P$ value of 0.059 [Table 1].

No significant difference was observed between Group 1 (i.e.) sound teeth at baseline and posttest with a $P$ value of 0.636. There was a significant difference within all the groups with $P$ value of 0.0001 after comparing between the baseline and the posttest [Graph 2].

Demineralized tooth compared with 5-ppm fluoridated milk and 5-ppm fluoridated deionized water showed an effect size of 2.15 and 2.87, with confidence interval (CI) of –26.8 to –8.1 and –36 to –17.3, respectively. It was found that Group 5, that is, 5-ppm fluoridated deionized water, had the highest of 40.46% gain in surface hardness followed by Group 4, that is, 5-ppm fluoridated milk with 17.26%, Group 3 plain milk with 13.73%. The hardness gained for Group 2 was –12.3 indicating surface hardness lost due to demineralization.

**DISCUSSION**

RC is an extensive problem for a high percentage of geriatric population. The various factors that influence are increase in longevity and more number of teeth, gingival recession with exposed root surfaces, xerostomia due to prescription of drugs in chronic
systemic conditions, and head and neck cancer patients with radiation treatment. Physical limitations all of these contribute in RC formation. The critical pH of dentine is 6.7, whereas that of enamel is about 5.2; therefore, root dentine will demineralize more easily in very weak acids and RC progresses double the rate of coronal caries. Thus, it is critical to go for preventive strategy as early as possible.\textsuperscript{[19]}

The reduction in caries prevalence over the last 30 years has been accredited to the universal use of fluoride.\textsuperscript{[20]}

\textbf{Figure 3:} pH cycling model (Adapted from Ivancakova \textit{et al.}\textsuperscript{[12]})

\textbf{Graph 1:} Postintervention surface microhardness (PISMH) comparison between interventional groups using analysis of variance (ANOVA) and \textit{post hoc} analysis
Fluoride is the most commonly used agent for healing of the initial demineralization process. The presence of fluoride in saliva and plaque, during a cariogenic challenge, can inhibit the dissolution of hydroxyapatite crystals and subsequently enhance remineralization.\[^{[21]}\] but additional fluoride applications by using mouth rinsing are also required in handling RC. Mouth rinses are well accepted and widely used in combination with dentifrices. Homemade rinses will be very handy for such purpose. In this study, fluoridated milk is used as a vehicle as it is easily accessible and also rich in calcium which could further enhance remineralization. The effectiveness of fluoridated milk on enamel remineralization has shown positive effect on caries reduction,\[^{[22-24]}\] whereas only few studies have determined the effect of fluoridated milk on root dentine remineralization.\[^{[8,11-13]}\]

Considering the limited research on the effect of root dentine remineralization on artificially induced caries lesions, this study was designed. This study is first of its kind to analyse 5 ppm of fluoridated milk and its effect on remineralisation using surface hardness test. However, comparison was made with other studies that use same materials or methods of analysis. The findings are similar to the study where significant differences were observed within the intervention groups, that is, plain milk and 5-ppm fluoridated milk, with respect to the decrease in caries lesion depth using polarized light microscopy and microradiography.\[^{[13]}\] The study showed that there was depletion of enamel porosity of 20% volume in 40-h remineralization of enamel in milk and also proved components of milk are capable of disseminating into the full depth of the demineralized enamel.\[^{[13]}\]

### Table 1: Post hoc comparison between the intervention groups

| Comparison between interventional groups | Mean Difference | 95% Confidence Interval | p value |
|-----------------------------------------|----------------|------------------------|---------|
| Group 1 Sound root                      |                |                        |         |
| Group 2 Demineralized root treated      |                |                        |         |
| with plain deionized water              |                |                        |         |
| Group 3 Demineralized root treated      |                |                        |         |
| with plain milk                         |                |                        |         |
| Group 4 Demineralized root treated      |                |                        |         |
| with fluoridated milk                   |                |                        |         |
| Group 5 Demineralized root treated      |                |                        |         |
| with fluoridated deionized water        |                |                        |         |

\(p\) value - 0.05* statistically significant  
\(p\) value - 0.001** statistically highly significant

Graph 2: Baseline and postintervention surface microhardness (PISMH) comparison within the interventional groups
body lesion causing changes in enamel pore volume and reducing the area of micro lesion. When fluoride was present in the milk, the depth reduction was seen to be almost twice as large in percentage. These results show a similar result to our study where there is a gain in hardness in both plain milk and one-third gain in 5-ppm fluoridated milk when compared with plain milk, whereas the gain is more than twice in 5-ppm fluoridated deionized water.

Another study showed similar finding with significant difference within the intervention groups, that is, plain milk, 2.5 ppm of fluoridated milk and 10 ppm fluoridated milk, with respect to the decrease in caries lesion depth using polarized light microscopy and microradiography.[10] The authors have explained that influence of plain milk showed only some remineralizing effect, whereas with 10 ppm of fluoride it is more effective.

Similar study showed contradictory result, where 5 ppm of fluoridated milk and plain milk used as a medium in biofilm caries model (streptococcus mutans); it showed decrease in mean surface hardness loss when compared to control but there was no significant difference between plain milk and 5 ppm of fluoridated milk.[11] The authors have explained it could be due to the less mineralized nature of dentine which showed nonsignificant result when compared to the control (plain milk).

Coronal caries lesions have a superficial layer which is well-mineralized, whereas such layer is not present in RC lesions, especially in root dentin it has foremost demineralized layer on the surface.[25,26] In natural RC lesions, a thin layer of cementum is usually present, which are hypermineralized superficially. In this respect, natural RC lesions can dissimilar from artificial carious lesions. In this study, the root cementum was mechanically removed before the experiments leading to root dentin as the surface zone subjected to demineralization, which does not mimic natural root surface is the limitation in the study. This limitation has occurred due to the standardization carried out to the experimental lesions which has to be compromised for better comparisons between groups.

The strength of the study is that demineralized/caries-induced surface can be compared with the natural RC based on the surface hardness. The demineralizing process, whether natural or artificial, produces 45% reduction in mineral. This loss ranges from 15% to 25%.[27] The surfaces of hardness lost is –12.3 and has represented almost natural demineralization of dentine. Hence, it is similar to the natural demineralization of root dentin.

**Conclusion**

Surface microhardness analysis revealed that plain milk, 5-ppm fluoridated milk, and 5-ppm fluoridated deionized water showed remineralizing potential on RC. Plain milk, 5-ppm fluoridated milk, and 5-ppm fluoridated deionized water showed an increase percentage of surface hardness gained indicating a positive remineralization effect on root dentin. 5-ppm fluoridated deionized water and 5-ppm fluoridated milk have a better remineralization effect compared to plain milk. However, both have a similar effect statistically.

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**Conflicts of interest**

There are no conflicts of interest.

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