Original Article

Effect of organic versus inorganic fluoride on enamel microhardness: An *in vitro* study

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Abstract

Introduction: Dental caries is one of the most prevalent infectious diseases affecting the human dentition. Fluorides are effective anti-carious agents and have been widely used for caries prevention in the form of systemic and topical fluorides. Neutral sodium fluoride (NaF) is commonly used as a topical fluoride agent. A special category of topical fluorides are organic fluorides in the form of amine fluorides (AmF). Researchers have reported that AmF is superior to inorganic fluorides in improving the caries resistance of enamel due to the significant anti-enzyme effect of the organic fragment.

Aim: The aim of the present study was to compare the enamel surface micro hardness after topical application of NaF and AmF solutions.

Materials and Methods: Twenty fresh samples of sound human enamel were treated with demineralizing solution for 72 h and divided into Group A (treated with NaF) and Group B (treated with AmF) solutions for 3 min twice daily for 7 days. In between treatment, the samples were stored in artificial saliva. The enamel surface hardness was measured with Vickers hardness test at baseline, post-demineralization and post-treatment with two different fluoride solutions (NaF and AmF) and a comparative analysis was made.

Results: The increase in mean micro hardness of human enamel after treatment with AmF application was found to be statistically significant ($P < 0.01$) when compared to the mean micro hardness after treatment with NaF.

Conclusion: Fluoride enhances the remineralization process by accelerating the growth of enamel crystals that have been demineralized. It can be concluded from the present study that AmF compounds result in a marked increase in enamel micro hardness when compared to NaF.

Keywords: Amine fluoride; dental caries; micro hardness; remineralization; sodium fluoride

INTRODUCTION

Dental caries is the most prevalent chronic infectious disease affecting the human dentition. It is currently recognized as a dynamic process since periods of demineralization alternate with periods of remineralization through the action of calcium, fluoride, and phosphorous present in the saliva.[1] It is, therefore, viewed as a biofilm induced disease cause by an imbalance in physiologic equilibrium between tooth mineral and biofilm fluid.[1]

The surgical approach to managing dental caries was developed a century ago as at that time there was no other valid alternative. Presently, advances in the field of caries research have led to improved understanding of the disease process. Now, early detection of initial carious lesions and emphasis on preventive measures holds the key to controlling dental caries.[2]

The discovery of the anti-cariogenic properties of fluorides is one of the most important landmarks in the history of dentistry.[3] Fluoride is the most commonly used remineralizing agent. The cariostatic effect of fluoride is primarily due to its ability to decrease the rate of demineralization by forming fluorhydroxyapatite and enhancing the remineralization of incipient carious lesions.[4]

Fluoride incorporated into the enamel mineral during tooth development has little effect on the caries process. It is the fluoride that is incorporated post-eruptively during the caries challenge that plays an important role in caries

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prevention. The most effective caries preventive fluoride regimen is provided by the daily application of topical fluoride in the form of dentifrices and mouth-rinses.[5]

The various types of topical fluorides used in dentistry are: Sodium fluoride (NaF), sodium mono-fluorophosphate, stannous fluorides and acidulated phosphate fluoride. All these fluorides are inorganic in nature and are available in the form of solutions, varnishes, foam, gels, dentifrices, etc.[3] Bioavailability of fluoride is an important factor in caries prevention. This depends on the solubility of the fluoride containing compound and its adhesion to the tooth surface.[6,7]

In 1957, Muhleman *et al*. found that organic fluoride like amino fluoride compounds were superior to inorganic fluorides in reducing the solubility of the enamel.[8] Subsequently, products containing amine fluorides (AmFs) were introduced and have gained popularity in Scandinavian countries. Research has demonstrated that AmF produces the most powerful enrichment of fluoride in enamel.[9,10] AmF has greater anti-cariogenic property for two reasons: (a) Presence of fluoride, (b) the amine (organic) component has an antiplaque effect inhibiting bacterial adhesion and tensioactive property which allows accumulation of fluoride close to the tooth surface providing a sustained fluoride release.[9,11]

Table 1: Materials used in the study

Materials	Composition
Sodium fluoride mouthwash 0.2% w/v (pH-5.51) (S-flo, Dr. Reddy's laboratory, Bangalore)	904 ppm of sodium fluoride
Amine fluoride (pH-4.70) (Amflor oral rinse, group pharmaceuticals, Bangalore)	480 ppm of amine fluoride
Demineralizing solution (pH-4.5) (group pharmaceuticals, Bangalore)	Calcium chloride, 2.2 mM sodium dihydrogen orthophosphate, 2.2 mM lactic acid
Artificial saliva (pH-6.71) (Cash pharmacy, Bangalore)	0.65 g/L potassium chloride British Pharmacopoeia (BP), 0.058 g/L magnesium chloride BP, 0.165 g/L calcium chloride BP, 0.804 g/L dipotassium hydrogen phosphate U.S. pharmacopeia, 0.365 g/L potassium dihydrogen phosphate, 2 g/L sodium benzoate, 7.8 g/L sodium carboxymethyl cellulose BP, deionized water to make 1 L

BP: British pharmacopoeia

Recently, AmF containing dentifrices and mouth-rinses have been introduced in India.

Hence, the aim of the present *in vitro* study was to compare the micro hardness of demineralized enamel after topical application of NaF and AmF solutions.

MATERIALS AND METHODS

Twenty intact and non-carious sound human premolars extracted from patients of age group 14-20 years for orthodontic purpose were collected and disinfected according to Occupational Safety and Health Administration (OSHA) recommendations. The teeth were decoronated at cement-enamel junction and sectioned mesio-distally into two halves using a high speed diamond disc. The resultant 40 samples were randomly divided into 2 groups-Group A ($n = 20$ samples) and Group B ($n = 20$ samples). The samples were mounted in cylindrical molds filled with self-cure acrylic resin and polished. A Vickers micro hardness (ZWIK/ROELL indentec, Japan) indenter (Vicker's Hardness (VH) indenter) was used to evaluate the baseline micro hardness under 100 g loads applied for 15 s at 5 different points each 1 mm apart and the mean was measured. Samples were stored in glass tubes containing 20 ml demineralizing solution for 72 h in an incubator at a temperature of 35°C. Following demineralization, surface micro hardness measurements were made using the VH indenter. The samples in Group A were immersed in NaF mouth wash (S-flo mouthwash, Dr. Reddy's laboratory, Bangalore) for 3 min twice daily for 7 days. The samples in Group B were immersed in AmF (Amflor oral rinse, Group pharmaceuticals, Bangalore) following the same protocol. In between treatment the samples were stored in artificial saliva [Table 1]. The enamel surface hardness in both groups was measured using Vickers hardness test after treatment and a comparative analysis was made.

Statistical analysis

Statistical analysis was done using Student *t*-test (STATA version 10.1, StataCorp LP, Texas, USA). Table 2 shows the comparison of micro hardness within each group.

RESULTS

The samples treated with NaF (Group A) demonstrated a slight decrease in the mean micro hardness after

Table 2: Comparison of micro hardness within each group

Time interval	Group	Mean	Standard deviation	SE of mean	Mean difference	*T*	*P* value
Baseline	A	460.43	7.98	2.52	−1.060	−0.309	0.761
	B	461.49	7.34	2.32			
Post demineralization	A	437.54	10.29	3.25	−1.280	−0.305	0.764
	B	438.82	8.41	2.66			
Post remineralization	A	448.70	14.50	4.59	−26.120	−3.678	0.002*
	B	474.82	17.15	5.42			

*denotes significant difference

treatment (from baseline). In contrast, the samples treated with AmF (Group B) demonstrated a statistically significant ($P < 0.01$) increase in mean micro hardness [Table 2]. Bar graph is shown in Figure 1.

DISCUSSION

The progression or reversal of dental caries depends upon the balance between demineralization and remineralization processes. This balance depends upon several factors like salivary calcium and phosphate concentration, bioavailability of fluoride and the pH of saliva.[12] Demineralization of enamel leads to the dissolution of hydroxyapatite and diffusion of calcium and phosphate ions toward the enamel surface.[13] One of the main reasons for enamel demineralization is undoubtedly the drop in pH below the critical point for hydroxyapatite dissolution.[14]

Remineralization occurs when the pH raises and calcium and phosphate from the saliva along with fluoride start forming new hydroxyapatite crystals on the enamel. The critical pH range for demineralization and remineralization is between 4.3 and 5.0.[12,15] Hyper-saturation of calcium and phosphate ions causes re-precipitation of hydroxyapatite forming an intact superficial layer on the enamel surface. Remineralization of enamel is enhanced by the presence of fluoride ions which leads to the formation of fluroapatite.[13]

Thus, fluoride plays an important role in the remineralization process. It acts as a catalyst and influences reaction rates with dissolution and transformation of various calcium phosphate minerals.[16] The released mineral ions are re-precipitated as fluoroapatite which is less soluble and provides additional protection onto the apatite crystals.[3,17]

Different fluoride formulations may have different effects on caries prevention. Dentifrices and mouth-rinses are popular topical agents. The use of mouth-rinses to deliver chemotherapeutic agents is well accepted.[18] It has been reported that use of fluoride mouth-rinses can lead to higher levels of oral fluoride retention than fluoride

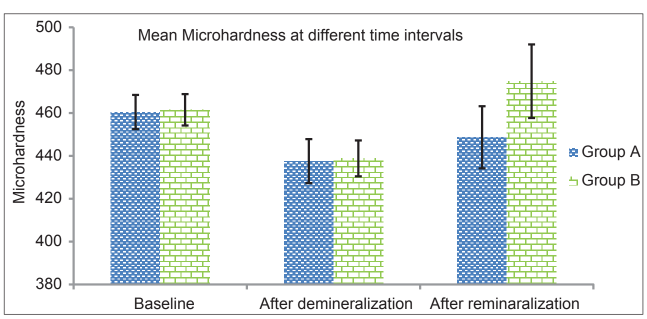

Figure 1: Bar graph showing the comparative micro hardness of group A and group B after demineralization and remineralization

dentifrices.[19] Hence, fluoride mouth-rinses were used in this study. The demineralization protocol was designed for 72 h, which was to simulate the duration that occurs in the oral cavity in caries prone individuals.

There are different methods for evaluation of demineralization and remineralization of enamel which may be direct or indirect. Direct techniques are longitudinal microradiography, transverse microradiography and wavelength independent X-ray microradiography. Indirect techniques include polarized light microscopy, quantitative energy dispersive X-ray analysis, micro hardness measurement methods and iodide permeability. Indirect methods are nevertheless quantitative and can measure changes in the real physical parameters. In case of polarized light they can detect the general porosity of the enamel substrate. The use of surface micro hardness tests can measure the change in surface structural strength.[20]

Surface micro hardness is a physical property which assesses the effect of chemical and physical agents on hard tissues of teeth. This is a useful way to examine the resistance of fluoride treated enamel.[21] It is an appropriate test for enamel due to its fine microstructure, non-homogenous and brittle nature. Micro hardness indentation provides a relatively simple, rapid and non-destructive method in demineralization and remineralization studies.[4] Micro hardness tests are of different type which includes: Knoop, Vickers and Brinnel. In the present study, VHN was adopted as the basis for investigation over Knoop's because the square shape of indent obtained in VHN is more accurate to measure. Even the minute changes in the square shape indent obtained after the test can be easily detected.[22]

The Vickers hardness values obtained during the baseline mean micro hardness measurements in the present study were in the range of 460.43-461.49 VHN. The surface mean micro hardness values for each group of the enamel specimens reduced to 437.54-439.82 VHN after the demineralization process for 72 h. After remineralization, the mean micro hardness in Group A increased to 448.70 VHN whereas in Group B it was 474.82 VHN.

The results of the present study reveal greater increase in mean micro hardness following remineralization with AmF than with NaF, which was statistically significant. This may be attributed to the beneficial properties of AmF. AmF is an organic compound-(N-octadecyltrimethylendiamine –N, N, N-tris (2-ethanol)-dihydrofluoride [$C_{27}H_{58}N_2O_3 2HF$]) consisting of two functional groups, that is: A cationic amino organic group and a bound ionic fluoride group.[23]

Several studies have reported the caries inhibitory effects of AmF.[9,11] It is a surface active agent having tensioactive and anti-glycolytic properties. The unique surface active property provides self-alignment of the hydrophobic part towards the oral cavity and the hydrophilic part towards the tooth surface which leads to an accumulation of fluoride close to the tooth surface.[9] The accumulated fluoride is available directly for the formation of calcium fluoride as a labile fluoride reservoir.[9,24] The benefits of AmF are many. The amino (organic) molecules readily bind to the enamel surface. Its surface active/tensioactive property leads to fast distribution of fluoride and homogenous coating on tooth surface for prolonged period. Monoamine aliphatic compounds offer protection to enamel against acid decalcification. The end result is the increased bioavailability of fluoride which plays a crucial role in preventing a net mineral deficit in enamel due to caries.

In case of Group A, there was a slight increase in surface hardness following remineralization, but it did not reach close to baseline levels. NaF which is inorganic in nature reacts with hydroxyapatite of enamel forming a thick layer of calcium fluoride. This thick layer of calcium fluoride interferes with further diffusion of fluoride from the topical fluoride thus providing a relatively lower bioavailability of fluoride ions.[25] Another limitation is that the sodium cation does not have any independent caries prophylactic property.

Thus, in terms of bioavailability this translates into a significantly higher salivary fluoride level being available from AmF than NaF. Studies have reported that the quality of remineralization with AmF is superior to that of NaF due to the slow release of fluoride and a constant salivary fluoride level.[26,27]

In contrast to our findings, a study by Lippert *et al* compared the anticaries potential of two new commercial dentrifices containing AmF and NaF by Vickers hardness testing. They concluded that NaF showed superior anticaries potential when compared to AmF. They attributed this to the presentation of the fluoride compound and formulation excipients on deciding the anti-caries potential *in vitro*.[28]

There are not many studies on enamel micro hardness to verify the efficacy of remineralization. However, several studies using polarized light microscopy, quantitative energy dispersive X-ray analysis etc., have shown the remineralizing potential of AmF.[27,29] Arnold *et al*., using polarized light microscopy had concluded that the superficial enamel layer were more stable after AmF application than after NaF or sodium monofluoride application.[27] Another study has also revealed that slightly acidified fluoride containing dentifrices like AmFs may have a marked effect on enamel remineralization.[29] Data from the literature has also shown that AmFs deposit more fluoride on enamel than sodium or stannous fluoride from concentrated topical fluoride preparations.[30-32]

An important factor contributing to the overall activity of topical fluorides is the mechanism of fluoride retention in the mouth and its subsequent clearance. The results of the present study as well as those from available literature indicate that organic fluorides like AmF are superior remineralizing agents than inorganic fluorides and hence must be more frequently used to combat dental caries. However, the present study is an *in vitro* one the results of which may be quite different from the dynamic process that occurs in the *in vivo* situation. Therefore, further *in vivo* studies are necessary on to validate the findings of the present study.

CONCLUSION

- Both the inorganic (NaF) and the organic fluoride (AmF) were effective in remineralization
- NaF remineralization did not restore the hardness of the enamel surface to that of pre-operative levels
- AmF provided greater benefits than NaF which was satistically significant
- The remineralized surface obtained by exposure to AmF was found to be harder than intact enamel.

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