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Original Article

Comparative assessment of the long-term effects of 5% sodium fluoride varnishes with and without tricalcium phosphate on enamel microhardness of primary teeth: An *in vitro* study

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ABSTRACT

Background: Dental caries is among chronic childhood diseases. Fluoride varnish application is a suggested modality, which can reinforce the enamel and decrease the risk of caries. This study aimed to assess the long-term effects of three commercially available fluoride varnishes with and without tricalcium phosphate on the enamel microhardness of primary teeth.

Materials and Methods: In this *in vitro* experimental study, 52 extracted primary molars were randomly assigned to four groups: no fluoride varnish application (control), and treatment with Ariadent, Parla, and Morvabon fluoride varnishes. After specimen preparation and artificial caries induction, the respective fluoride varnish was applied, and the teeth were subjected to remineralization-demineralization cycles at 37°C. The enamel microhardness was measured at baseline, after artificial caries induction, and 24 h and 45 days after the fluoride varnish application. The trend of change in the mean microhardness of the study groups was analyzed by repeated measures ananlysis of variance (alpha = 0.05) and Bonferroni correction. In the next step, the trend of change in microhardness (mean difference) was compared among the groups by the Tukey's HSD test at a 0.05 level of significance.

Results: Enamel microhardness increased during the study period in all groups ($P < 0.05$), except for the control group. Intergroup comparison of long-term effects revealed that the increase in microhardness in the Parla group was significantly greater than that in the Ariadent ($P = 0.01$) and Morvabon ($P = 0.006$) groups.

Conclusion: The results of this *in vitro* study indicated that application of Parla fluoride varnish, which contains tricalcium phosphate, increased the primary enamel microhardness, and had a more durable effect over time compared with the other two varnishes.

Key Words: Deciduous, dental caries, dental enamel, hardness tests, primary prevention, tooth

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INTRODUCTION

Dental caries is among the most common oral diseases of childhood, which is considered as a serious public health dilemma.[1,2] Dental caries often occurs due to an imbalance between the demineralization and remineralization cycles in the

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enamel following the formation of microbial biofilm on the tooth surface and acid production over time.[3] Proper oral hygiene and a healthy diet are the main requirements for the prevention of microbial biofilm formation, and caries progression. However, in high-risk individuals, application of remineralizing agents may be recommended as an adjunct. Fluoride is among the most commonly used remineralizing agents. The optimal clinical efficacy of fluoride for caries cessation, especially in the enamel, has been well documented.[3]

Among the fluoride products, application of 2.26% sodium fluoride (NaF) varnish (containing 22600 ppm fluoride) has been recommended in children under 6 years of age at high risk of caries.[4] A recent clinical study and a meta-analysis of clinical studies indicated the optimal efficacy of fluoride varnishes for caries prevention.[5-7] Comparison of the efficacy of NaF varnishes with other fluoride-containing products has shown the comparable or superior efficacy of NaF varnishes.[8-12] However, some other studies reported that application of NaF varnishes alone did not increase the enamel microhardness.[13] Accordingly, researchers tried to compare the efficacy of different NaF varnishes. Some short-term studies assessed the increase in fluoride uptake by the enamel, or increase in microhardness of incipient enamel lesions over a 7–10-day period.[14-18] A long-term study evaluated fluoride release from the enamel surface over 6 months.[19] Considering the shortcomings of previous studies on the long-term efficacy of different NaF varnishes, this study aimed to assess the primary enamel microhardness following the application of three 5% NaF varnishes with and without tricalcium phosphate (commonly used in Iran), over a long period of time.

MATERIALS AND METHODS

This experimental study was approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.DENTISTRY.REC.1403.009). The sample size was calculated to be 15 teeth in each group according to a study by Virupaxi *et al*.,[19] using one-way ANOVA feature of Minitab 18 software, and assuming alpha = 0.05, beta = 0.20, the largest mean difference between the groups to be 55, and similar standard deviations of the groups equal to 46.80.

A total of 52 primary molars that had been extracted due to dental abscess and had a sound buccal surface with no hypocalcification, fracture, or crack were collected. The teeth were immersed in Chloramine T solution for the purpose of disinfection. After disinfection, they were stored in distilled water to preserve their moisture, and prevent structural changes. Next, the roots were cut 1 mm below the cementoenamel junction, and the crowns were mounted in acrylic resin, such that the sound buccal surface remained exposed for testing. To achieve a smooth surface for the microhardness test, the buccal surface of the teeth was wet-polished with rough-(800-grit), medium-(1000-grit), and fine-grit (1200-grit) abrasive papers.

To measure the enamel microhardness, the standard Vickers test was performed in a Vickers hardness tester (160V-Test II; Baresiss, Germany). In this test, a 300 g load was vertically applied to three points on the buccal surface with a minimum of 100 μm distance from each other for 15 s. The mean of the three values was calculated and recorded as the baseline microhardness of the teeth (Vickers hardness number). To induce artificial caries, the teeth were immersed in a demineralizing solution with a pH of 5 for 10 h followed by immersion in a solution with a pH of 4.5 for 15 h. The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 0.05 M acetic acid; the pH was adjusted to 5 using 1 M KOH. The composition of solution and its duration of use were determined in a pilot study, which showed that the abovementioned condition led to development of white spot lesions on primary enamel and decreased the microhardness by approximately 30%–40%. The microhardness was measured again after demineralization.

The teeth were then randomly assigned to four groups. Group 1 served as the control group with no fluoride varnish application. Ariadent varnish (Asia-chemi Teb company, Tehran, Iran) was applied in group 2, Parla varnish (Parla company, Tehran, Iran) was applied in group 3, and Morvabon varnish (Morvabon company, Tehran, Iran) was applied in group 4. In groups 2–4, the specimens were dried with an air spray, and the respective NaF varnish was applied on their buccal surface by a microbrush in two coats. The specimens were subsequently immersed in artificial saliva as the remineralizing solution, composed of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl; the pH was adjusted to 7.0. After 24 h, the residual fluoride was removed by a soft toothbrush in the fluoride varnish

groups. The microhardness was then measured again by an examiner blinded to the group allocation of the specimens. Next, the specimens were subjected to remineralization-demineralization cycles (pH cycling) for 45 days, such that the specimens were immersed in the demineralizing solution for 3 h, and the remineralizing solution for 21 h within each 24 h. The specimens were kept in an incubator at 37°C during the entire study period.[20] After 45 days, the microhardness of the specimens was measured again by a blind examiner.

Normal distribution of data was evaluated by the Shapiro–Wilk test. The trend of change in the mean microhardness over time was analyzed by repeated measures ANOVA, considering the type of varnish as between-subject variable (alpha = 0.05). The Bonferroni correction was used for multiple comparisons in the next step. The trend of change in microhardness (mean difference) was compared among the groups by Tukey's HSD test using SPSS version 26 at a 0.05 level of significance.

RESULTS

Table 1 presents the measures of central dispersion for the microhardness of the study groups. The mean baseline microhardness was measured to be 349.48 ± 39.75, which was within the normal range reported for sound primary enamel.[21] The results showed no significant difference in baseline microhardness among the study groups ($P = 0.941$). This finding indicated that the baseline microhardness of all groups was comparable, with no significant difference. As shown in Table 1, the Parla group showed the highest percentage of increase in microhardness during the time interval between demineralization and 24 h and 45 days after the varnish application, followed by the Ariadent and Morvabon groups. The control group showed the smallest increase in microhardness after demineralization.

Within-group comparisons

Repeated measures ANOVA was applied to analyze the effect of time on microhardness as the dependent variable. The results showed a significant change in microhardness over time in all groups ($P < 0.001$ for all). In all 4 groups, the mean microhardness significantly decreased after demineralization compared with baseline ($P < 0.001$ for all). The mean microhardness at 24 h after varnish application significantly increased compared with postdemineralization in all groups ($P < 0.001$ for all). The mean microhardness at 45 days remained unchanged compared with after demineralization in the control group ($P = 0.084$), but significantly increased in all three fluoride varnish groups ($P < 0.001$).

Between-group comparisons

Pairwise comparisons by the Tukey's HSD test were performed to analyze the effect of varnish type on microhardness as the dependent variable.

Comparison of the groups at 24 h revealed a significant difference in the trend of change in microhardness among them from the demineralization time to 24 h after varnish application ($P < 0.00$). The results revealed a significantly greater increase in microhardness in the Parla and Ariadent groups compared with the control and Morvabon groups. The percentage of microhardness change was significantly greater in the Parla group compared with the control ($P < 0.001$) and Morvabon ($P = 0.01$) groups. The percentage of microhardness change in the Ariadent group was significantly higher than that in the control group ($P < 0.001$), and was insignificantly higher than that in the Morvabon group ($P = 0.98$). Microhardness changes were not significantly different between the control and Morvabon groups ($P = 0.54$).

Comparison of the groups at 45 days revealed a significant difference in the trend of change in microhardness among them from the demineralization time to 45 days after varnish application ($P < 0.001$). The Parla group still showed the highest mean change

Table 1: Measures of central dispersion for the microhardness (Vickers hardness number) of primary enamel in the study groups

Study groups	Study phases, minimum–maximum, mean±SD			
	Baseline	Demineralization	24 h after fluoride application	45 days after fluoride application
Control	269–472, 346.36±43.267	106–258, 200.95±32.211	153–382, 223.56±53.476	189–392, 251.90±48.631
Ariadent	259–397, 349.82±32.600	150–288, 199.31±38.406	210–320, 259.62±27.543	214–343, 268.18±30.757
Parla	288–401, 349.77±31.195	160–288, 216.18±30.268	236–370, 298.95±33.860	261–407, 327.79±39.560
Morvabon	265–453, 352.00±50.210	120–290, 222.79±38.295	200–352, 271.77±39.533	201–389, 297.85±45.131

SD: Standard deviation

in microhardness, which was significantly greater than that in the control ($P < 0.001$), Ariadent ($P = 0.01$), and Morvabon ($P = 0.006$) groups. The Ariadent, Morvabon, and control groups had no significant difference with each other in the percentage of change in microhardness ($P > 0.05$).

According to the results, Morvabon varnish caused no significant change in microhardness, and the efficacy of Parla varnish for the enhancement of microhardness of primary enamel was significantly greater than that of Ariadent and Morvabon varnishes [Table 2, Figures 1 and 2].

DISCUSSION

Noninvasive treatments and preservation of tooth structure are major priorities in contemporary dentistry. It appears that remineralization of demineralized tooth structure should be prioritized when encountering incipient enamel caries. Accordingly, calcium, phosphorous, fluoride, and Vitamin D are supplied for the tooth structure.[22]

The fluoride content of drinking water may not be sufficiently high; therefore, application of topical fluoride products has been approved, among which, NaF varnish has been recommended for children under 6 years of age. Evidence shows that 5% NaF varnish contains 22600 ppm fluoride. It adheres to the tooth surface for a couple of hours, and serves as a source of sustained fluoride release during this period. The fluoride release rate from this product is influenced by the varnish formulation and the matrix used, its mechanism of binding to insoluble NaF, its interactions with the tooth structure, and environmental factors such as the salivary flow rate, saliva calcium and phosphate contents, consumption of acidic beverages, and activity of oral bacteria. This topic has been investigated through assessment of the degree of enamel demineralization and remineralization by chemical analysis, evaluation of the hardness of tooth sections, and microradiography after the application of fluoride varnish. The present study assessed the efficacy of three fluoride varnishes available in the Iranian market by evaluating their effects on enamel microhardness. Microhardness measurement is a simple, noninvasive, and fast method for this purpose. It is reproducible, and allows repeated microhardness measurements of the same specimen at different time points. It also possesses optimal accuracy and reliability. Featherstone[23] (1999) and Lata *et al*.[24] stated that enamel microhardness measurement is an efficient method for evaluation of enamel mineral loss or gain. Since formation of hydroxyapatite crystals and recording the microhardness change need time, in the present study, the microhardness of the specimens was evaluated both in the short-term (after 24 h) and also after 45 days, which is longer than the assessment time in previous studies for evaluation of the durability of the effect of varnishes.

The present results showed that Ariadent and Morvabon varnishes improved primary enamel

Table 2: Mean percentage of microhardness change in the study groups at 24 and 45 days compared with after demineralization

Study Groups	24 h after fluoride application, mean±SD	45 days after fluoride application, mean±SD
Control	14.45±23.55	27.94±28.49
AriaDent	33.49±21.14	37.85±21.55
Parla	40.10±19.25	53.05±22.38
Morvabon	24.58±23.30	35.91±20.43

SD: Standard deviation

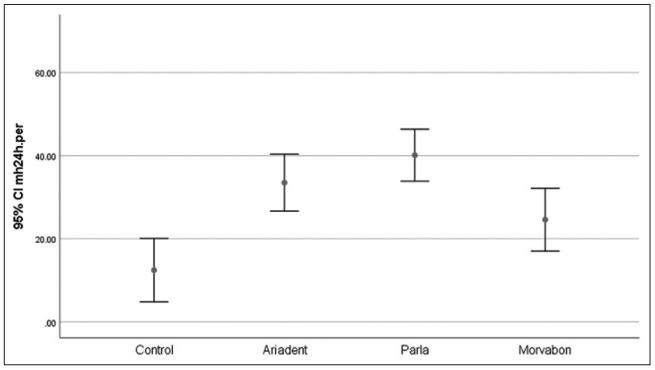

Figure 1: Error bar of the mean and 95% confidence interval of microhardness change in the study groups 24 h after varnish application.

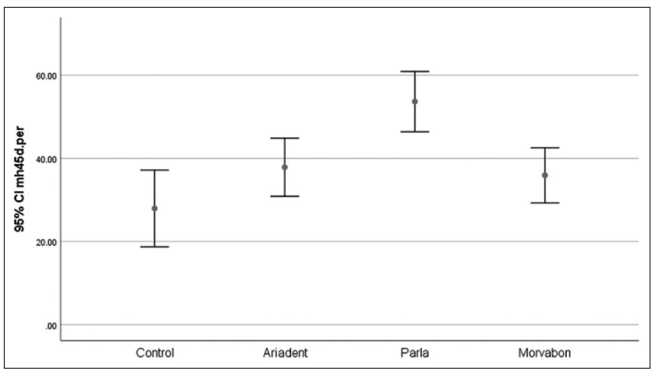

Figure 2: Error bar of the mean and 95% confidence interval of microhardness change in the study groups 45 days after varnish application.

microhardness, but their effects did not last for 45 days. However, Parla varnish increased enamel microhardness in the short-term, and its effect lasted for 45 days.

In a short-term study on primary teeth, Jafari *et al*.[14] evaluated fluoride uptake by primary enamel after the application of fluoride varnishes by using the acid-etch enamel biopsy method. They reported the lowest fluoride uptake in the Ariadent group, and the highest in the Sultan and Voco groups. Nonetheless, they added that fluoride uptake was more homogeneous in the Ariadent group, and stated that this varnish is reliable and applicable. In the present study, data dispersion was acceptably low in the Ariadent group.

Some similar short-term studies were conducted on permanent teeth. Molaasadolah *et al*.[15] evaluated the enamel microhardness of permanent premolars following the application of Ariadent and Duraflor varnishes in 10 days under pH-cycling. They showed a significantly greater increase in microhardness in both fluoride varnish groups compared to the control group. The other studies on fluoride varnishes conducted within a 1-week period showed that Fluor Protector[16] and Enamelast[18] optimally increased the microhardness, and Duraflor and Enamel Pro[17] effectively enhanced enamel resistance to demineralization.

Virupaxi *et al*.[19] (2016) evaluated the long-term effects of three varnishes on fluoride release over 6 months, and reported that application of Clinpro™ XT on the primary tooth surface resulted in continuous fluoride release over a 6-month period.

NaF varnishes have a low free fluoride content, which is released following saliva penetration into the varnish matrix. Therefore, they need a longer exposure time to the saliva. Aside from the exposure time, difference in the type of matrix and mechanism of binding or dissolution of NaF in the matrix affect the efficacy of the varnish.[2] Accordingly, the manufacturers of fluoride varnishes need to provide a suitable protocol regarding the application time of their products.

Some previous studies focused on the frequency of effective use of NaF varnish to find the most efficient frequency of use. An *in vitro* study indicated higher enamel microhardness in the group that received NaF varnish 4 times a year compared with once and twice a year, and the intensive method of 4 times/week.[25] Salehzadeh *et al*.,[26] Farhadian *et al*.,[27] and Sivapriya *et al*.[28] used NaF varnish with a higher frequency rate in the short-term and reported its improved efficacy for remineralization of demineralized permanent enamel. Some other studies also confirmed the optimal clinical efficacy of varnish application three times within a 2-week period and four times within 4 weeks to control white spot lesions.[29,30]

Some short-term studies indicated that a fluoride varnish containing tricalcium phosphate had a superior remineralizing capacity than a varnish without tricalcium phosphate, and suggested using an additive such as tricalcium phosphate to improve the protective ability of varnishes on primary enamel.[31,32] A 30-days *in vitro* study conducted to assess the long-term effectiveness of fluoride varnish containing tri-calcium phosphate on permanent teeth confirmed the advantages of these products[33] and was in accordance with the results of the present study on primary teeth.

According to the results obtained in this *in vitro* study under a controlled environment, the Parla varnish had a longer remineralizing effect on primary enamel. Unlike Ariadnet and Morvabon, the Parla fluoride varnish contains tricalcium phosphate, which can increase and prolong the remineralizing effect of fluoride. It appears that the manufacturers of less effective NaF varnishes need to recommend a 3-time application protocol within a 2-week period to maximize fluoride uptake and enamel microhardness.

In the present study, pH-cycling (demineralization-remineralization cycles) was performed over a longer period of time (45 days) instead of one week to assess the durability of the effect of fluoride varnishes on primary enamel in a setting simulating the oral environment. The artificial saliva and solutions were refreshed daily during this period to prevent the adverse effect of materials released from the tooth structure on the varnish. The fluoride content of the pH-cycling solutions mimics the role of fluoride in the human saliva or the fluoride obtained from foods and drinks. Nonetheless, it should be noted that many influential factors present in the oral environment, such as oral microorganisms, differences in chemical composition of the saliva in different individuals, and oral hygiene practices, cannot be simulated *in vitro*; therefore, *in vitro* results cannot be completely generalized to the clinical setting.

Future studies are recommended to assess the efficacy of the available varnishes with different

timing protocols. Furthermore, fluoride release and fluoride uptake should be evaluated in addition to microhardness.

CONCLUSION

The results revealed that the Parla varnish, containing tricalcium phosphate, increased the primary enamel microhardness and had a more durable effect than other tested varnishes. Considering the observed differences in the durability of the effects of different fluoride varnishes, the timing protocol for clinical application of each varnish needs to be further evaluated, and revised, if required.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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