Original Research
Cariology

Acceptability and effect of TiF_4 on dental caries: a randomized controlled clinical trial

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Abstract: This randomized three-armed controlled clinical trial compared the effect of titanium tetrafluoride (TiF_4) and sodium fluoride (NaF) varnishes on caries control in smooth surfaces of permanent dentition and children's acceptability. Sixty children (6-8 y/o) were randomly divided into TiF_4 (2.45% F^-), NaF (2.26% F^-) or placebo (control) groups. Varnishes were applied on permanent teeth once a week for the first 4 weeks and after the 6th and 12th months of the study. The variables were as follows: International Caries Detection and Assessment System (ICDAS) scores, quantitative fluorescence changes, visual plaque index (VPI) and degree of acceptability. Two-way RM-ANOVA, ANOVA/Tukey and $\chi 2$ tests were performed ($p < 0.05$). No differences were found between the treatments with respect to ICDAS scores ($p = 0.32$). Only TiF_4 reduced the mean fluorescence loss significantly at 18 months compared to the baseline ($p = 0.003$). TiF_4 showed a lower percentage of new caries lesions by tooth surface than the placebo, while NaF did not induce such a change ($p < 0.014$). Regardless of the treatment, more than 95% of the participants reported being satisfied. For all groups, the VPI decreased significantly at 3 months compared to the baseline value ($p < 0.001$), with no differences between the treatments ($p = 0.17$). TiF_4 had a similar ability to control caries lesions as NaF; however, only TiF_4 differed from the placebo ($p = 0.004$). The acceptability of TiF_4 varnish was similar to that of NaF varnish.

Keywords: Clinical Trial; Dental Caries; Fluorides.

Introduction

The protective effect of titanium tetrafluoride (TiF_4) on dental caries has been intensively investigated using *in vitro* and *in situ* models.[1-6] Comar et al.[3] demonstrated a significant effect of TiF_4 varnish in the remineralization of initial enamel caries lesions *in situ*, regardless of caries activity, while NaF was able to remineralize enamel lesions under low cariogenic challenges only. The mechanism of action of NaF varnish is based on calcium fluoride (CaF_2) deposition on the dental surface, which acts as a fluoride reservoir interacting with tooth hydroxyapatite and as a mechanical barrier against acids.[7,8] On the other hand, TiF_4 has an additional effect due to the presence of titanium that reacts with apatite-forming compounds such as hydrated titanium phosphate and titanium dioxide, responsible for the highly

acid-resistant layer precipitated on teeth, improving the mechanical barrier.[8] Furthermore, TiF_4 varnish induces a higher deposition of CaF_2 on enamel due to its low pH than that induced by NaF varnish.[8]

The mechanical barrier created by fluoride protects enamel against demineralization induced by bacterial acids. Furthermore, the fluoride reservoir can speed up remineralization, inducing the growth of fluor-hydroxyapatite-like crystals. In the case of TiF_4, the reaction of titanium with phosphate from apatite forms a glaze-like layer on the enamel surface, and may improve its mechanical resistance.[8,9] Due to this latter property, TiF_4 varnish can be considered a good option for the control of noncavitated enamel caries lesions, especially on smooth surfaces, avoiding progression and, consequently, the need for microinvasive approaches.[10]

There are few clinical studies testing the anti-cariogenic or remineralizing effect of TiF_4 solution,[11,12] but none have evaluated TiF_4 when it is included in a varnish. Previous studies showed a superior effect of TiF_4 varnish compared to TiF_4 solution and/or NaF varnish on enamel caries lesions *in situ*,[2,5] which justifies the efficacy of this study and emphasizes its contribution to the previous literature. Despite its low pH, TiF_4 varnish has shown similar levels of toxicity on murine fibroblast lineage (NIH/3T3) and gingival fibroblasts compared to NaF varnish.[5,13,14] However, we have no information about the degree of patients' acceptability of this new product.

Therefore, the aim of this randomized, three-armed, controlled, longitudinal and double-blind clinical trial was to compare the effect of 4% TiF_4 varnish with a commercial 5% NaF varnish (gold standard) and a placebo varnish (negative control) on the control of enamel caries lesions in smooth surfaces of children's permanent dentition residing in an optimally fluoridated area by using International Caries Detection and Assessment System (ICDAS) and quantitative light fluorescence (QLF) tools. Furthermore, the children's degree of acceptability of this new product was analyzed. The tested null hypotheses were that there would be no significant difference between fluoride varnishes in: a) the prevention and/or b) regression/progression of noncavitated enamel caries lesions in permanent teeth and c) the degree of children's acceptability.

Methodology

Clinical procedures

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This clinical trial was registered in the Brazilian Clinical Trials Registry (identifier RBR-5vwj4y), approved by the local ethics committee (Number: 59787116.2.0000.5417) and by the Municipal Secretariat for Education of Bauru (São Paulo, Brazil). Five municipal schools were selected from the 4 most populous regions of the city (Figure 1). The parents/guardians of each child participant provided signed written informed assent. The children also provided a signed consent form with age-appropriate language.

Sixty healthy children (6–7 years old) from 5 public schools from Bauru (Brazil), an area optimally fluoridated (0.8 ppm F), were selected according to the study inclusion and exclusion criteria.[15,16]

The mean of the ICDAS index and the children's sex were considered for the allocation of the children in one of the three groups, for each school, independently (n = 20/per treatment in total); the three groups were 4% TiF_4 varnish (2.45% F, pH 1, FGM-DentsCare, Joinville, SC, Brazil); 5% NaF varnish (2.26% F, pH 5, Duraphat®, Colgate-Palmolive Co., New York, NY, USA) or placebo varnish (negative control, without F, pH 5, FGM-DentsCare, FGM-DentsCare, Joinville, Brazil). The sample calculation (10 per group) was obtained based on a previous clinical trial[12] (decayed, missing or filled tooth surface—DMFS—number after a period of 2 years: 1.94 ± 0.36 for TiF_4 solution and 2.60 ± 0.54 for acidulated phosphate fluoride—APF) considering a dropout of 30%, statistical power of 85% and an alpha-error of 5%.

The primary outcomes measured in this study were a) the reversal or progression of active enamel lesions on smooth surfaces using ICDAS and QLF parameters after 18 months and b) the prevention of new carious lesions evaluated by ICDAS after

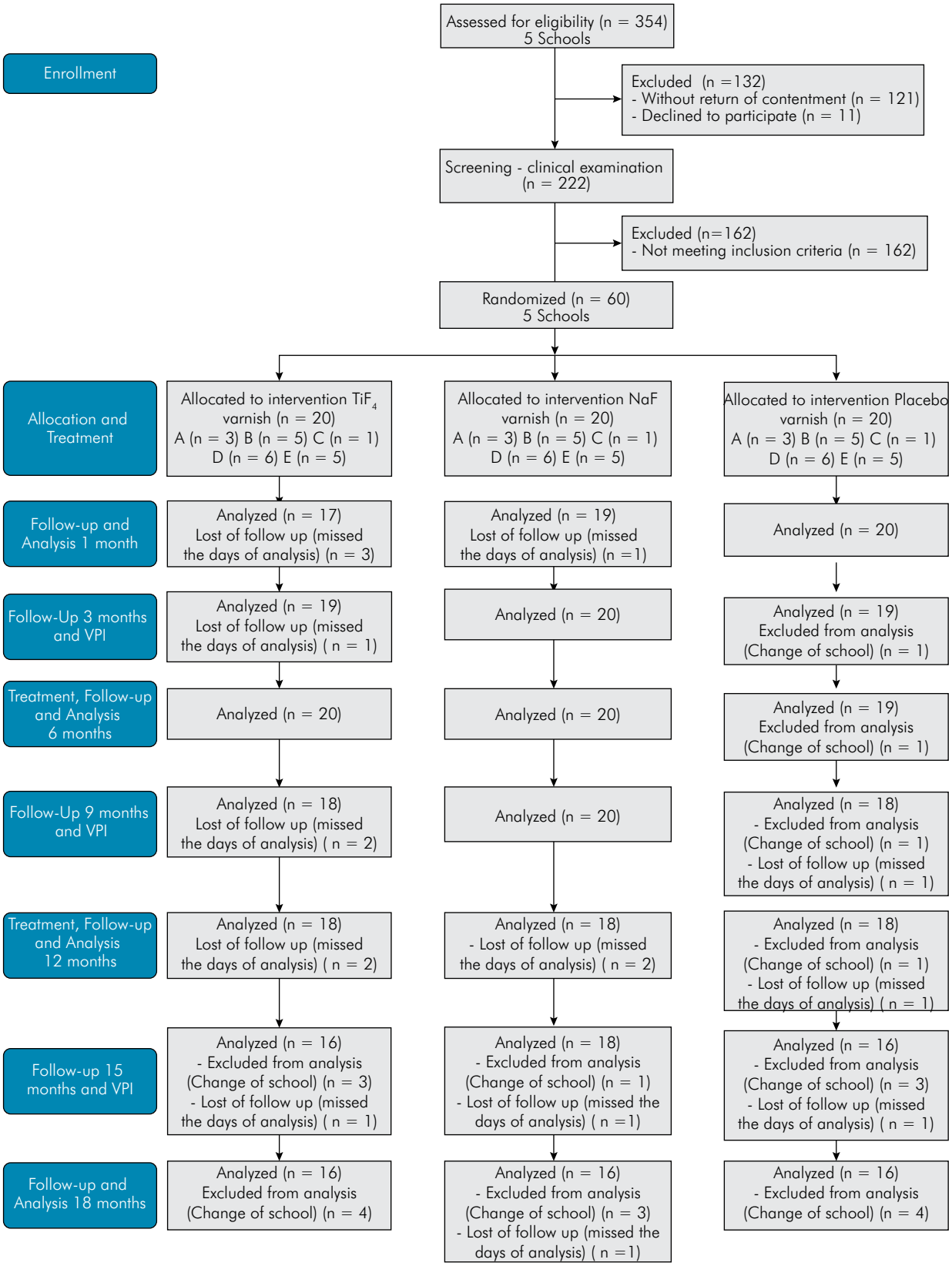

Figure 1. CONSORT flow diagram of the study. School distribution: A.– Northern Region 1; B.– Northern Region 2; C. Southern Region; D. Western Region; E. Eastern Region.

18 months. The secondary outcomes were a) patient satisfaction with and acceptability of the intervention assessed using a visual scale and b) the reduction in visible plaque during the study.

Varnish was applied on all smooth surfaces of permanent teeth once a week for 4 consecutive weeks,[16] with a single application at the 6th and 12th months of the study.[17,18] The children, their parents/guardians and the researchers who performed the clinical examination and QLF analysis were blinded to the treatment.

The degree of acceptability was evaluated after each varnish application through a visual scale.[16] Since the degree of acceptability remained constant, the percentage of children who contributed to each score was calculated by a mean of the six visits.

The clinical examination was performed[16] by two trained examiners (inter- and intraexaminer agreement, kappa > 0.8). Both the ICDAS index evaluation and QLF analysis were performed at the beginning and at the end of the study, while QLF analysis was also performed 1 month after treatment and at the 6th and 12th months of the study. The DMFS score was utilized for additional data (as described in the ReBEC), but it was not included as an outcome, considering that DMFS is not a method applied for identifying carious lesions at a very early stage.[19]

The QLF system (Inspektor Research Systems BV, Amsterdam, The Netherlands) measures the fluorescence loss of noncavitated enamel carious lesions and can assess lesion regression or progression over time.[16] The area of the lesion (mm^2) and the mean fluorescence loss (ΔF, %, detection threshold of 5%) were determined by QLF 2.00f software (Inspektor Research System BV, Amsterdam, The Netherlands).[16]

During visits to the schools, children were educated with respect to cariogenic diet and oral hygiene. The visual plaque index (VPI)[20] was assessed before oral hygiene procedures (conducted under supervision by using the oral hygiene kit of the study: fluoride toothpaste with 1450 ppm F as MFP, a toothbrush and dental floss) for all tooth surfaces at the 3rd, 9th and 15th months of the study. A score of 0 was equivalent to the absence of dental plaque, and a score of 1 was equivalent to the presence of dental plaque. The VPI (number of surfaces that were given a score of 1) was converted into the percentage of surfaces with visible dental plaque, also called the full mouth plaque score (FMPS).

Statistical analysis

Intention-to-treat analysis was used, where all randomized participants were included in the statistical analysis and compared according to the group to which they were originally assigned. Considering that missing values represented less than 20% of the sample, the average of the other two treatments was used to compensate for missing data, which provided a conservative estimate, as suggested by Spineli et al.[21] The number of smooth surfaces was considered for ICDAS analysis, while the number of children was used for the statistical analysis of QLF, satisfaction degree and FMPS data. The data were compared via GraphPad Prism version 7.0 software for Windows (GraphPad Software, San Diego, USA) with a level of significance < 5%.

The numbers of smooth surfaces that had ICDAS scores from 0-6 from each group were compared by ANOVA. With respect to both prevention and progression/regression of preexisting lesions, $\chi 2$ was used to check the association between ICDAS distribution (progression/regression) and the type of treatment. The $\chi 2$ test was performed considering the whole population and for each region of the city separately. The values of QLF parameters (mean per child) and FMPS (% tooth surface per child) were compared using 2-way ANOVA/Tukey's test. With respect to prevention, the percentages of dental surfaces per child that had a score of 0 at baseline and a score > 0 at the end of the study were compared using ANOVA/Tukey's test. The association between the degree of acceptability and the type of treatment was determined using the $\chi 2$ test.

Results

The number of children selected and examined during the entire study is described in the flowchart following CONSORT guidelines (http://www.consort-statement.org) (Figure 1). During the follow-up and at the end of the study, we lost a maximum of 4 participants per group (within 20%).

From 60 children (females, n = 24 and males, n = 36), 128 smooth surfaces (5.2% of the total surfaces [n = 2,481 surfaces]) presented ICDAS scores of 2 (active lesions) at baseline (TiF_4 n = 45; NaF n = 41; placebo n = 42). No differences in the ICDAS score distribution were found among the groups at baseline and at the end of the study (ANOVA, $p > 0.05$, Table 1).

Thirteen percent of children presented caries lesion progression (increasing from a score of 2 to scores of 5–6) at the end of the study, according to the following distribution: TiF_4 (n = 2), NaF (n = 4) and placebo (n = 2) varnish groups. Notably, 13% of children, corresponding to 2, 3 and 3 children in the TiF_4, NaF and placebo groups, respectively, exhibited regression (decreasing from a score of 2 to scores of 0–1). To be considered to exhibit regression, the patient should have at least one lesion with a lower ICDAS score and no lesions with progression at the end of the study. Children who presented lesion progression or new cavitated lesions received appropriate treatment. For most children, the lesions did not present clinical changes during the study period, regardless of the treatment.

When the tooth surface was considered and the sound surface was included in the analysis, the rate of progression was 9x higher than the rate of regression for all groups, but "no progression/regression" was still the most prevalent observation (Table 1). An association was observed between the caries lesion response per tooth surface (progression/regression) and the type of treatment ($\chi 2$, $p < 0.0001$, Table 1). Higher progression (3x) and regression (3x) were observed in the placebo group than in the fluoride groups. When the city regions were considered separately, the Northern Region was the only region showing a significant association (Table 1, $p < 0.0001$).

For QLF analysis, no significant differences were found among the treatments with respect to the lesion area (WS) and mean fluorescence loss (ΔF). When the periods of analysis were compared within each treatment group, only the TiF_4 group had a lower ΔF mean after 18 months than at baseline (2-way ANOVA, $p = 0.0003$, Table 2).

With respect to the prevention of new lesions, the TiF_4 group presented a significantly lower percentage of sound surfaces (with a score of 0 at baseline) affected by caries per child at the end of the study compared to the placebo group, while the NaF group did not differ from either of the other groups (TiF_4 and placebo) after 18 months of follow-up (Figure 2, ANOVA $p = 0.015$).

Regardless of the treatment group, more than 95% of children were very pleased or pleased with the treatment. None reported a score higher than 4. No association was found between the degree of acceptability and the type of treatment ($\chi 2$, Figure 3, $p > 0.05$). No patient reported side effects during the study.

No differences were found among the groups with respect to FMPS (2-way ANOVA, $p > 0.05$). There was a significant reduction in visible plaque (%, $p < 0.0001$) in all groups at the 9th month (TiF_4: 35.1 ± 29.2; NaF: 30.0±30.9 and placebo: 22.8 ± 17.3%) and 15th month (TiF_4: 21.9 ± 17.5; NaF: 28.3 ± 27.0 and placebo: 22.3 ± 16.2%) compared to the 3rd month (TiF_4: 58.3 ± 34.1; NaF: 65.8 ± 34.7 and placebo: 64.4 ± 33.4%), with no significant difference between the last 2 months (9th and 15th months).

Table 1. Numbers of tooth surfaces (all smooth surfaces of permanent teeth) with progression, regression or no progression/regression after 18 months of treatment with TiF_4, NaF or placebo varnish for the total population and by city region.

Variable	General			North			South			East			West		
	TiF_4	NaF	Placebo	TiF_4	NaF	Placebo	TiF_4	NaF	Placebo	TiF_4	NaF	Placebo	TiF_4	NaF	Placebo
Progression	16	21	58	9	9	37	0	0	0	4	7	9	3	5	12
Regression	2	2	6	1	1	4	0	0	1	0	0	0	1	1	1
No changes*	819	791	766	268	280	222	44	37	43	202	159	191	305	315	310
p-value	< 0.0001			< 0.0001			p = 0.395			p = 0.312			p = 0.166		

*The surface was still sound (score 0) or presented no progression or regression, in case of scores > 0 at baseline. $\chi 2$ results showed a significant association between the lesion response and the type of treatment for the total population and for the Northern Region only.

Table 2. Mean ± S.D. of the data obtained by QLF at baseline and after 1, 6, 12 and 18 months of treatment with TiF_4, NaF or placebo varnish.

Variable	Baseline	1 month	6 months	12 months	18 months
WS (mm^2)					
TiF_4	3.4 ± 1.5	3.8 ± 2.6	3.8 ± 1.8	3.8 ± 1.8	3.6 ± 2.4
NaF	3.7 ± 1.9	3.6 ± 2.1	4.0 ± 1.9	3.9 ± 1.5	3.6 ± 1.4
Placebo	4.1 ± 1.9	3.5 ± 1.9	3.6 ± 1.7	4.0 ± 1.9	3.6 ± 1.6
ΔF (%)					
TiF_4	-17.5 ± 3.9^a	-16.7 ± 3.6^a	-16.1 ± 3.0^{ab}	-16.3 ± 3.2^{ab}	-14.6 ± 4.0^b
NaF	-15.7 ± 3.2^a	-15.3 ± 3.1^a	-16.4 ± 2.7^a	-15.9 ± 2.2^a	-14.9 ± 2.2^a
Placebo	-16.4 ± 3.2^a	-16.2 ± 3.6^a	-14.5 ± 1.6^a	-15.5 ± 1.6^a	-14.4 ± 2.0^a

Two-way RM-ANOVA (WS: time p = 0.555 and treatment p = 0.971; ΔF: time p = 0.0003 and treatment p = 0.327). Different lowercase letters indicate significant differences among times within each treatment group (n = 20 children/group).

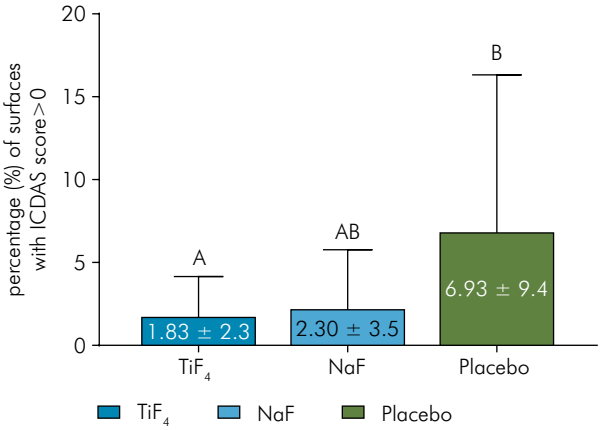

Figure 2. Mean and standard deviation of percentage of dental surfaces per child who previously had an ICDAS score of 0 that developed caries lesions at the end of study (18 months). ANOVA/Tukey (p = 0.015, n = 20 children/group)

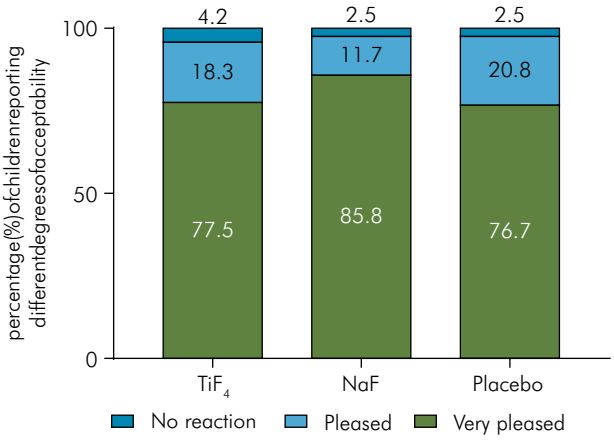

Figure 3. Mean percentage of children reporting different degrees of acceptability at the end of the six applications of the varnish using a visual scale. No child scored > 4. $\chi 2$ results showed no association between the type of treatment and the degree of acceptability (p = 0.515, n = 20 children/group).

Discussion

All tested null hypotheses were accepted since no differences were found between the TiF_4 and NaF varnishes for the analyzed parameters. At the patient level, no benefit of fluoride varnish application could be seen; however, when considering the tooth surface, some benefit was shown, since the numbers of lesions that progressed were lower for both fluoride groups than for the placebo group, as shown in the Northern Region of the city (a socially disadvantaged region). Therefore, we can infer that the effect of the fluorides evaluated in our study may have been influenced by the caries risk of the population (associated with socioeconomic status), as previously discussed.[22]

On the other hand, for the QLF analysis, TiF_4 improved the amount of mineral gain over time, resulting in a significant reduction in enamel fluorescence loss after 18 months of study, in agreement with previous *in vitro* and *in situ* studies.[1-6] Although the results were statistically significant for the TiF_4 group, the difference compared to the other groups was too small, which may not have clinical relevance. The different results between the clinical and complementary methods are due to the sensitivity; small mineral changes can be quantified by QLF, but they may not be clinically detectable.

With respect to the prevention of new caries lesions, the TiF_4 group also presented a significantly lower percentage of previously sound surfaces affected by caries at the end of the study compared to the placebo group, but the percentage was not different from the NaF group. Despite modest

findings, this positive finding may be explained by the reaction of TiF_4 with apatite, which produces an acid-resistant layer and allows the incorporation of Ti and F into enamel, making it more resistant to bacterial acids.[8]

Previous clinical trials have tested the effect of TiF_4 as a solution,[11,12] showing promising effects on the prevention of demineralization and the improvement of remineralization. An annual application of 1% TiF_4 significantly reduced the appearance of new lesions in permanent teeth (33% reduction) compared to 1.25% acidulated phosphate fluoride (APF) in a follow-up of 3 years.[11] In our study, the difference between TiF_4 and NaF was approximately 21.7% for new lesions per tooth (n.s.) after 18 months, a value slightly lower than previous findings.[11]

Pomarico et al.[12] demonstrated that permanent teeth treated with 4% TiF_4 solution (once) plus MFP toothpaste (daily) for 4 weeks had significantly lower lesion areas (74.5% reduction in lesion size) than teeth treated with MFP toothpaste only (67%). In our work, TiF_4 reduced the mean fluorescence loss by 16.6% after 18 months compared to the baseline value. The low value of the % caries reduction found in our work may be due to the low caries risk level of our studied population. Furthermore, previous work used scanning electronic microscopy to measure the lesion area and included only 8 patients, a very low number; therefore, the data from that work cannot be extrapolated to the clinic.[12]

On the other hand, NaF did not have a protective or remineralizing effect on enamel compared to placebo varnish in our study. Some clinical studies evaluating the potential of biannual NaF varnish applications to prevent dental caries in primary teeth were unable to find significant differences between the fluoridated and nonfluoridated groups (or just brushing) after 24 months of follow-up.[18,22,24] However, Arruda et al.[25] demonstrated that school children, who had their permanent teeth treated with 5% NaF varnish (biannual), had a 41% reduction in caries increment (new lesions) compared to placebo after 12 months of follow-up. Compared to our study, it is clear that the protective effect of NaF found by the cited authors[25] was due to the high caries risk level of their population (DMFS 5.9) compared to that of our children (DMFS 0.05, cavitated lesion was found only in occlusal surfaces).

In a longer follow-up period (26 months) with biannual application of NaF varnish, no differences were found in caries incidence on the first permanent molar of children treated with NaF varnish (16% children) compared to placebo varnish (19%),[26] similar to our findings. In agreement, Milson et al.[27] also demonstrated no difference in DFS increment for patients treated with NaF varnish (annual application) and placebo after 36 months of follow-up. Accordingly, the above-cited works also tested the effect of NaF in children at low risk for caries. On the other hand, for primary dentition, even in high-risk populations, fluoride varnish fails to reduce caries development in toddlers.[18]

Marinho et al.[28] suggested that fluoride varnishes have good protective potential, regardless of the frequency of application (two or four times a year); however, the quality of evidence is still moderate. Moreover, the authors could not demonstrate the influence of external factors on the effect of fluoride varnishes with respect to caries control (such as exposure to other fluoride sources).[28] In our work, all children were exposed to fluoridated water (0.8 ppm F) and toothpaste (1450 ppm F, as MFP). The region showing higher caries lesion progression was the Northern Region (a socially disadvantaged region), where we found some protective effect of fluoride varnishes.

The cited systematic review also showed that the side effects of and information on the acceptability of fluoride varnishes were inconclusive because these details were often not reported in clinical trials.[28] Therefore, our study provides new and very important information indicating that fluoride varnishes are very well accepted by children, regardless of the type of fluoride salt used. No participant reported tooth staining or any other side effects due to fluoride varnish application. This finding is very interesting since we expected that TiF_4, due to its low pH, could cause an unpleased taste change, as reported in a recent *in situ* study testing mouthwashes.[29] Considering that the varnish is applied in low amounts on tooth surfaces, with little direct contact with soft tissues, the eventual taste change may be reduced. As varnish is applied

every 6 months, the risk of tooth staining is rather low compared with the daily use of a TiF_4 mouthwash.[30]

The beneficial effect of NaF varnish in remineralization is more often reported for primary teeth than for permanent dentition.[31,32] However, there is still no consensus on the frequency of application (to stop or reverse noncavitated lesions on smooth surfaces), varying the application every 3 to 6 months,[27,33] or using one application per week for 4 consecutive weeks.[16,31] We followed the last protocol to improve remineralization, as done by Almeida et al.,[16] and we further reapplied the varnishes every 6 months to achieve the preventive effect.[17,18]

Our study did not show a remineralizing effect of NaF, similar to what was found by Güçlü et al.,[31] except for the population of the Northern Region. The finding supporting the main hypothesis and justifying the low effect of NaF varnishes may be the low caries incidence rate found in the population. Hummel et al.[34] showed that the rate of caries progression is proportional to the severity, with a mean DMFS increment of 0.11 a year, suggesting longer follow-up periods for permanent teeth (> 36 months).[34]

Biofilm control is another important factor that could have influenced our results.[28] Our study showed improvement of oral hygiene after 3 months of study due to the frequent presence of researchers at schools encouraging children to practice better brushing habits. This finding might have contributed to the low caries progression and the lack of the fluoride varnish effect. The authors suggest that future studies should be done in high caries-risk populations (older children) to validate the effect of TiF_4 varnish in the worst scenarios.

Conclusion

This study shows that, under very well-controlled conditions, caries progression is low even after 18 months of follow-up; therefore, the effect of fluoride treatment is limited. TiF_4 and NaF varnishes exhibit similar behavior in this model, but TiF_4 varnish led to a slight improvement in remineralization and preventive effects compared to placebo varnish; however, its clinical relevance may be questionable. Its acceptability by children is similar to that of NaF varnish.

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