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Effect of dentifrices with different pH and fluoride concentrations on fluoride levels in biofilm and nails: an RCT

Fernanda de Araújo Trigueiro CAMPOS[a]
Aparecida Tharlla Leite de CALDAS[a]
Carlus Alberto Oliveira dos SANTOS[a]
Silmara Andrade SILVA[a]
Andre Parente de Sá Barreto VIEIRA[a]
José Maria Chagas VIANA FILHO[a]
Marília Afonso Rabelo BUZALAF[b]
Fábio Correia SAMPAIO[c]
Cristiane de Almeida Baldini CARDOSO[d]

[a]Centro Universitário de João Pessoa - Unipê, Department of Dentistry, João Pessoa, PB, Brazil.

[b]Univesidade de São Paulo – USP, School of Dentistry of Bauru, Department of Biological Sciences, Bauru, SP, Brazil.

[c]Universidade Federal da Paraíba – UFPB, Department of Clinical and Social Dentistry, João Pessoa, PB, Brazil.

[d]Universidade Cruzeiro do Sul, Department of Dentistry, São Paulo, SP, Brazil.

Declaration of Interests: MAR Buzalaf declares that University of São Paulo has a patent request in Brazil (INPI) for "Low-fluoride acidic liquid dentifrice and its use."

Corresponding Author:
Cristiane de Almeida Baldini Cardoso
E-mail: crisabc83@gmail.com

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Abstract: Purpose: to evaluate the effect of dentifrice pH and fluoride concentration ([F]) on fluoride uptake on the biofilm and nails of children from a non-fluoridated area. Methods: two hundred and twenty-eight two- to four-year-old children were randomly allocated into 3 groups according to the type of dentifrice: G1: 1100 µg F/g, pH 4.5 (n = 76); G2: 750 µg F/g, pH 4.5 (n = 74); and G3: 1100 µg F/g, pH 7.0 (n = 78). Nails were collected at 4, 8, and 12 months after starting dentifrice use and biofilm was collected 5 and 60 minutes after toothbrushing. The concentrations of F in nails and biofilm were analyzed by HMDS facilitated diffusion. Data were analyzed by Kruskal-Wallis/Mann-Whitney's test and the comparison between biofilm collection times was done using Wilcoxon test ($p \leq 0.05$). Results: a significant reduction of [F] in biofilm was observed 60 minutes after toothbrushing, regardless of the dentifrice used. However, 5 minutes after toothbrushing, G1 had a significantly higher [F] compared to G2 and G3, and 60 minutes after toothbrushing, [F] was significantly higher for G1 and G2 compared to G3. G1 and G3 had significantly higher [F] in the nails compared to G2. Conclusion: a lower dentifrice concentration is a relevant factor for the reduction of excessive fluoride intake. The use of a low-F acidified dentifrice combines the reduction of fluoride uptake with caries prevention by leading to greater incorporation of F into the biofilm over time.

Keywords: Dentifrices; Fluorides; Nails; Biofilms; Dental Caries; Randomized Controlled Trials as Topic.

Introduction

Fluoride (F) toothpastes are the most widespread method of topic fluoride use in the world and have played an essential role in reducing caries prevalence in recent centuries.[1-4] Data from the literature show that even when water fluoridation was discontinued in some cities or countries, caries decline persisted, especially due to the widespread use of fluoride.[2,5] According to a systematic review, in Brazil, this decline was more pronounced in 12-year-old children in the 2000s.[6]

Despite its benefits against dental caries, the cumulative fluoride (F) intake can be a risk factor for dental fluorosis in early childhood. Continuous use of regular dentifrices (1,100 mgF/g) by preschool children strongly contributes to daily F ingestion dose, especially in fluoridated communities.[7,8] As an alternative, the use of low-F (around 500 mgF/g)

dentifrices could decrease this ingestion. However, there are still uncertainties about the effectiveness of low-F dentifrices compared to regular ones (around 1000 mgF/g).[9-11]

A recent systematic review with meta-analyses showed that 1,500 μg F/g fluoride toothpaste significantly reduces caries increment compared to non-fluoride toothpaste use in primary dentition (MD -1.86 dfs, 95% confidence interval (CI) -2.51 to -1.21; 998 participants, one study, moderate-certainty evidence). However, caries-preventive effects are similar when comparing a 1055 μg F/g with a 550 μg F/g fluoride toothpaste (MD -0.05, dmfs, 95%CI -0.38 to 0.28; 1958 participants, two studies, moderate-certainty evidence). The findings of this review also show that toothbrushing with 1,450 μg F/g fluoride toothpaste leads to a slightly reduced decayed, missing and filled teeth (dmft) compared with 440 μg F/g fluoride toothpaste (MD -0.34, dmft, 95%CI -0.59 to -0.09; 2362 participants, one study, moderate-certainty evidence).[12]

Strategies have been proposed to increase the anti-caries efficacy of low-F dentifrices such as pH reduction.[13-15] Clinical trials have tested an experimental low-fluoride (500 mgF/g) acidic (pH 4.5) dentifrice. The first was conducted with 4-year-old children for 20 months, but only considered rates of caries progression (dmfs),[16] while the second included 2–4-year-old children for 12 months, considering lesions' activity.[17]

The results from these studies showed that low-F acidic dentifrice resulted in similar rates of caries progression (dmfs) and net increment as regular dentifrice. The "drop" technique was used to apply the dentifrice to the toothbrush,[18] reducing the amount of F swallowed, consequently decreasing F concentrations in the nails' in both studies.[16,17] It was suggested that nail F concentrations lower than 2 μg/g, which was found in children using the 550 mgF/g acidic dentifrice, were correlated with the absence of dental fluorosis in the permanent dentition[19]. Besides, the clinical efficacy of topical fluoridated products is directly related to the daily F levels of intraoral reservoirs as dental plaque, which can release small amounts of F when there is a reduction of pH over time.[20] The low pH of the dentifrice led to an increased F uptake by plaque in both clinical trials, which might be one possible hypothesis for the higher anti-caries potential of acidic dentifrices.[16,17,21]

It must be highlighted that the previous clinical trials were conducted in fluoridated communities and that the presence of fluoride in drinking water may have resulted in less detectable clinical differences among F concentrations of dentifrices. Thus, the present clinical trial aimed to evaluate the effect of low-F (750 μg/g) acidic, regular fluoride acidic (1,100 μgF/g), and regular fluoride neutral (1,100 μgF/g) dentifrices on fluoride uptake by biofilm and nails of 2–4-year-old children living in a non-fluoridated area.

Methodology

Experimental design

This randomized, double-blind clinical trial was approved by the Institutional Review Board of João Pessoa University (UNIPÊ) (167/2014 CAAE: 25024114.0.0000.5176) and was registered at http://clinicaltrials.gov/ (NCT03761485) (03/12/2018). It involved 2–4-year-old children from three public primary schools in João Pessoa, Brazil (non-fluoridated area). The sample size was selected based on the criterion of permanent residence, that is, the children must be permanent residents in the city to avoid bias regarding water supply fluoridation. The sample size calculation was performed based on a pilot study conducted with 168 children. Considering 80% power, 20% beta error, and 5% of alpha error, a final sample of 228 children was obtained.

Children were randomly assigned to three subgroups according to the type of dentifrice they were asked to use for a period of 12 months. The study design was a cluster-randomized controlled trial with schools as the unit of randomization (Figure 1). This assignment was done by one of the researchers (F.C.S.) by applying an algorithm previously established. The Excel software generated random numbers ranging from 0 to 99. Each of the three clusters was randomly assigned to 1 of 3 dentifrices: G1: 1100 μg F/g, pH 4.5 (regular F acidic); G2: 750 μg F/g, pH 4.5 (low F acidic); and G3: 1100 μg F/g, pH 7.0 (regular F neutral). The number of children allocated in subgroups 1, 2, and 3 was 76, 74, and 78.

Children of both sexes who were regularly enrolled in one of the three daycare centers and whose parents/caregivers consented to participate by signing the informed consent were included. The age range of 2 to 4 years was chosen as this is the critical period for the development of fluorosis as children ingest more toothpaste.

Exclusion criteria were children who had participated in other studies in the previous 3 months, children who did not allow clinical examination at school, children with orthodontic appliances, and children with extensive caries lesions with dentin sensitivity. Children who did not reside in the city of João Pessoa from birth to 2 years of age were also not included due to fluoridation of public water supply, as well as children whose caregivers (over 18 years of age) did not participate in the study guidelines for oral hygiene procedures or who withdrew from participation for any reason.

Fluoride concentration and pH of the experimental formulations, manufactured by Oralls Ind. Com. Ltda (São José dos Campos, Brazil), were stable

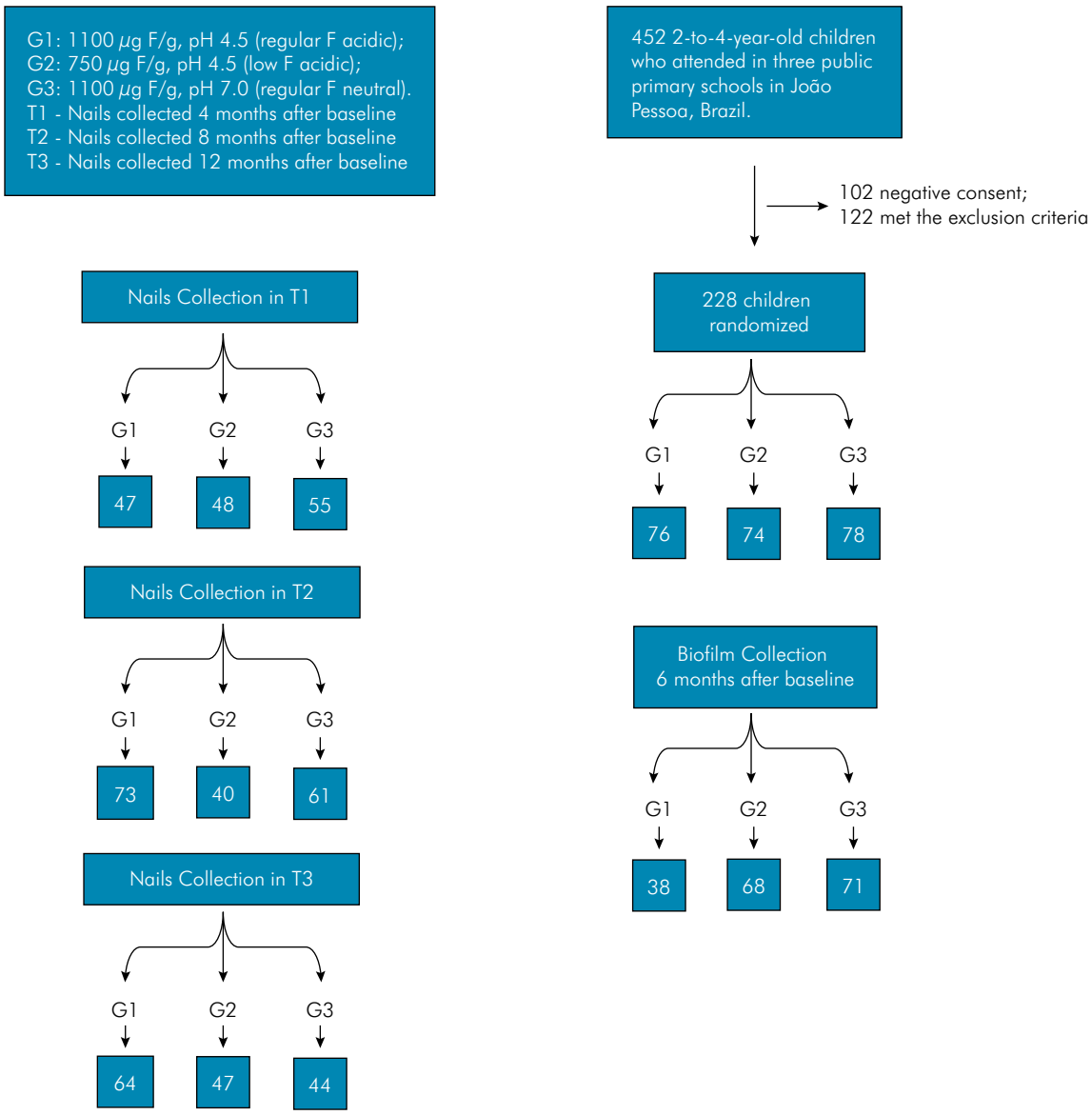


Figure 1. Experimental design of the study

(25°C) until the end of the experimental period. All formulations were NaF-based and identical, except for the dentifrices' color. The color code was kept by a person who was not involved in the examination of the children. Toothbrushes were supplied by Bitufo™ (Hypermarcas, São Paulo, Brazil) without conflict of interest. At baseline, children with restorative needs were treated at Public Health Centers or at University Dental Clinics of João Pessoa-PB.

Study regimen

School principals, teachers, and children's parents were given instructions about the study regimen. Kits containing a toothbrush that was marked with each child's name and the fluoridated dentifrice were supplied to schools. Kits were also delivered to all family members every 3 months to guarantee the use of the experimental dentifrices by the children, facilitating the compliance with the study protocol. The kits contained 4 toothbrushes, 4 dentifrice tubes (120 g each) according to the randomly selected group, and a leaflet on oral hygiene care. Parents and teachers received instructions regarding brushing frequency and time according to the study regimen.[17] The dentifrice was applied to the toothbrush using the "drop" technique (around 0.15 g).[18]

Biofilm collection and analysis

Biofilm was collected from a sample of 177 children participating in the study who had been using the dentifrices for 6 months. On the day before biofilm collection, parents/caregivers were instructed to brush only the occlusal surfaces of the teeth in order to allow biofilm accumulation on buccal surfaces. Biofilm samples were collected 6 months after baseline, and toothbrushing was performed at school by the researcher on the day of collection. Biofilm was collected according to a previous protocol[21]. After collection, Eppendorf tubes with the dental biofilm were frozen until analysis. Biofilm samples were dried for 2 h at 95°C and weighed, and F concentrations were determined after overnight hexamethyldisiloxane-facilitated diffusion,[22] as modified by Whitford[23,24]. The amount of F found in total biofilm was divided by biofilm weight (mg/kg, dry weight) and expressed as mmol/kg.

F concentration in nails

Nails were collected 4, 8, and 12 months after starting dentifrice use (T1, T2, and T3) in 150, 174, and 155 children, respectively. Parents received instructions and the children's nails were collected and prepared as previously described[17]. Fluoride concentrations were determined in the same way as described for biofilm and were expressed as mg/kg.

Statistical analysis

The software GraphPad InStat version 3.0 (GraphPad Software, La Jolla, USA) and SPSS Statistics 17 (Statistical Package for the Social Sciences) for Windows were used. Data were checked for normality and homogeneity using Kolmogorov-Smirnov and Bartlett tests, respectively. Biofilm data was not normally distributed, thus non-parametric tests were used. Different collection times were compared with Wilcoxon test and dentifrices data were compared with Kruskal Wallis/Mann-Whitney's test. Data of nails in T1, T2 and T3 were analyzed by ANOVA/Tukey's test. When data were analyzed together (considering the [F] of the three time-points), Kruskal-Wallis and Mann-Whitney tests were used. In all cases, the significance level was set at 5%.

Results

Of the initial sample of 228 children (120 boys and 108 girls; mean age 3.1 years, SD 0.81), biofilm was collected from only 177 children (38, 68 and 71 for groups 1, 2, and 3, respectively), representing a loss of 22% of the children who received the kits. The reasons for this loss were the absence of the children from school at the time of collection or lack/small amount of biofilm, which made F quantification impossible.

The collection of nails after 4 (T1), 8 (T2), and 12 (T3) months of dentifrice use was possible in 150, 174, and 155 children, representing a loss of 34, 24, and 32%, respectively, from the children who initially received the kits. Therefore, the sample was not homogeneous during collection periods because children missed school on the day of collection or were transferred to another school.

A significant difference in total plaque F concentration was found both among the dentifrices and between

time-points. A significant reduction of biofilm [F] was found at 60 minutes after toothbrushing, regardless of the dentifrice used. However, 5 minutes after toothbrushing, G1 had a significantly higher biofilm [F] compared to G2 and G3 ($p = 0.022$), and 60 minutes after toothbrushing, [F] was significantly higher for G1 and G2 compared to G3 (Table 1) ($p = 0.015$).

A significant difference in nail [F] was found between groups in T1 and T2 (Tables 2 and 3), but not in T3 (Table 4). The longitudinal evaluation of the same individuals at the three time-points (T1, T2, T3) showed that despite individual variations, these values did not vary significantly (Table 5). However, the sum of nail [F] of the three time-points was significantly higher in G1 and G3 than in G2 (Figure 2).

Table 6 shows the distribution (relative frequency) of children at risk of developing fluorosis based on a fluoride concentration in nails above 2 mg/kg (T1, T2, and T3).

Discussion

This clinical trial was designed to emphasize the importance of selecting a dentifrice with [F] to young children by balancing the best combination of anti-caries effect and risk of dental fluorosis, especially in non-fluoridated areas. According to a systematic review,[12] brushing with 1,500 µg F/g toothpaste reduces decayed, missing, and filled surfaces (dmfs) increment in primary dentition compared with

Table 1. Mean±SD and measures of dispersion of fluoride concentrations in dental biofilm (mmol/kg, dry weight), 5 and 60 minutes after the last brushing with the dentifrices with 1,100 µg F/g pH 4.5; 750 µg F/g pH 4.5 and 1,100 µg F/g pH 7.0.

Variables	G1: 1100 µg F/g acidic dentifrice (n = 38)		G2: 750 µg F/g acidic dentifrice (n = 68)		G3: 1100 µg F/g neutral dentifrice (n = 71)	
Time	5 min	60 min	5 min	60 min	5 min	60 min
[F] mean ± SD	1.15 ± 1.29^{A}	0.78 ± 0.70^{B}	1.04 ± 1.82^{A}	0.84 ± 1.29^{B}	0.99 ± 1.89^{A}	0.50 ± 0.52^{B}
Variance	1.68	0.50	3.34	1.68	3.58	0.28
95%CI	(0.77–1.59)	(0.59–1.03)	(0.64–1.47)	(0.55–1.19)	(0.63–1.47)	(0.39–0.64)
Median	0.73	0.63	0.46	0.38	0.40	0.34

Different superscript capital letters indicate a significant difference of the mean fluoride concentration between the first (5 min) and the second time-point (60 min) when the same dentifrice was used. A matched comparison was conducted between 5 min x 60 min after the last brushing with the same dentifrice (within-group comparison). Wilcoxon Signed Ranks Test, $p < 0.05$.

Table 2. Mean concentrations ± SD (mgF/Kg) of fluoride in nails of children who used different dentifrices at the first time-point (T1).

Type of dentifrice	n	Mean (SD)	Confidence interval (95%CI)	Min–Max
1,100 µg F/g acidic	47	3.55 ± 1.49^{a}	3.11–3.99	0.66–6.52
750 µg F/g acidic	48	2.94 ± 1.24^{b}	2.58–3.30	0.66–5.35
1,100 µg F/g neutral	55	2.97 ± 1.22^{a,b}	2.64–3.30	1.25–6.34
Total	150	3.14 ± 1.34	2.92–3.36	0.66–6.52

Different superscript lower-case letters indicate significant differences between groups (differences in columns) (ANOVA with Tukey-Kramer post-test, $p = 0.042$).

Table 3. Mean ± SD concentrations (mgF/Kg) of fluoride in nails of whcildren who used different dentifrices at the second time-point (T2).

Type of dentifrice	n	Mean (SD)	Confidence interval (95%CI)	Min–Max
1,100 µg F/g acidic	73	3.44 ± 1.75^{a,b}	3.11–3.78	0.94–7.58
750 µg F/g acidic	40	2.85 ± 0.89^{b}	2.57–3.14	1.68–5.68
1,100 µg F/g neutral	61	3.66 ± 1.54^{a}	3.26–4.05	1.09–7.87
Total	210	3.39 ± 1.58	3.18–3.61	0.94–7.87

Different superscript lower-case letters indicate significant differences between groups (differences in columns) (ANOVA with Tukey-Kramer post-test, $p = 0.040$).

Table 4. Mean ± SD concentrations (mgF/Kg) of fluoride in children's nails, who used different dentifrices at the third time-point (T3).

Type of dentifrice	n	Mean (SD)	Confidence interval (95%CI)	Min–Max
1,100 ppmF acidic	64	3.02 ± 1.48[a]	2.65–3.39	0.12–6.21
750 ppmF acidic	47	2.61 ± 0.99[a]	2.31 –2.90	0.10–3.93
1,100 ppmF neutral	44	2.94 ± 1.54[a]	2.47–3.41	0.72–6.45
Total	155	2.87 ± 1.37	2.65–3.09	0.10–6.45

Different superscript lower-case letters indicate significant differences between the groups (differences in columns) (ANOVA).

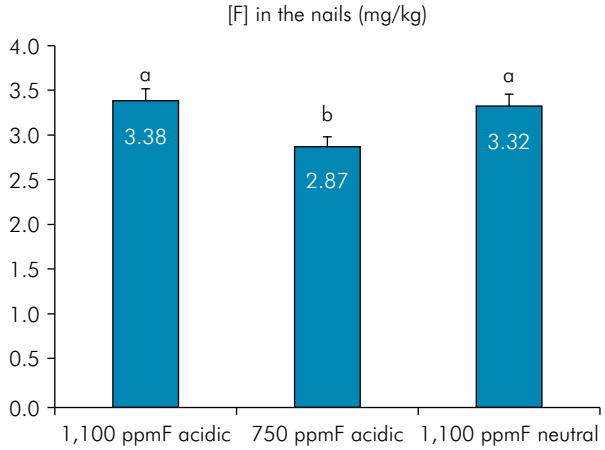
Table 5. Mean ± SD concentrations of fluoride in nails of children who used different dentifrices in the three time-points (T1, T2 and T3).

Type of dentifrice	n	T1	T2	T3	p-value*
1,100 µg F/g acidic	19	3.43 ± 1.38	3.50 ± 1.92	2.60 ± 1.53	NS
750 µg F/g acidic	14	2.40 ± 1.14	2.77 ± 0.57	2.58 ± 0.86	NS
1,100 µg F/g neutral	14	2.81 ± 1.04	3.27 ± 1.22	2.55 ± 1.36	NS
Total	47	2.94 ± 1.27	3.22 ± 1.43	2.57 ± 1.28	NS

Repeated measures ANOVA. There were no differences between means within the groups (horizontal evaluation). NS = Not significant.

Table 6. Distribution (relative frequency) of children at risk of developing fluorosis, considering a fluoride concentration in nails above 2 mg/kg (T1, T2 and T3).

Type of dentifrice	T1	T2	T3	Min-Max
	%	%	%	
1,100 µg F/g acidic	74.5	72.5	71.9	71.9–74.5
750 µg F/g acidic	70.8	80.0	66.0	66.0–80.0
1,100 µg F/g neutral	67.3	83.6	61.4	61.4–83.6
Total	70.7	77.1	67.1	67.1–77.1



Bars indicate the total mean of the three data collections with standard deviations. The comparison was carried out using the Kruskal-Wallis test (K = 8.734, p = 0.013) and the Mann-Whitney's U post-test for individual comparisons.

Figure 2. Mean fluoride concentrations in the nails (mg/kg) of the 12-month experimental period with three types of dentifrice with different fluoride concentrations and pH [1100 µg F/g pH 4.5 (n = 184); 750 µg F/g pH 4.5 (n = 135); 1100 µg F/g neutral (n = 160)].

placebo toothpaste, but caries preventive effects for 550 µg F/g and 1,055 µg F/g toothpastes are similar, although with a moderate level of certainty.

The benefit of the acidic dentifrice to prolong fluoride concentration in the biofilm until 60 minutes after brushing was achieved with the 750 µg F/g dentifrice in this study, corroborating the data of a randomized clinical trial.[21] In that trial, the acidic dentifrice (550 µg F/g) maintained a higher concentration of F in the biofilm compared with the dentifrice containing 550 µg F/g and with the dentifrice with neutral pH, similar to the dentifrice with 1,100 µg F/g and neutral pH. The values of F concentration in that study[21] were very similar to the values of F concentration in the present study. This effect was possibly due to the additional effect of water fluoridation in the city of Bauru-SP.

These findings also corroborate those of Kondo et al.,[25] where the [F] in biofilm after the use of low pH dentifrices was significantly higher than in biofilm after the use

of dentifrices with neutral pH. According to another study,[26] a dentifrice with a lower pH also leads to an increase in saliva F concentration after brushing, which may reflect in increased F concentrations in the biofilm. Moreover, according to another study,[21] fluoride at a low pH (~ 4.5) binds more easily to the *S. mutans* present in the biofilm than at neutral pH, functioning as a fluoride reservoir in the bacterial wall. Considering that biofilm is one of the most important F reservoirs in the oral cavity[27] and that individuals, especially children, do not completely remove it when brushing their teeth, a greater incorporation of F by acidified toothpaste can be considered an excellent mechanism of F release during a cariogenic challenge, even when lower [F] dentifrices (550 or 750 ppm) are used.

This is the first longitudinal study of fluoride exposure analyzing nails for biomarkers. A significant difference was found between groups in T1 and T2, but not in T3. However, considering the mean [F] of the three time-points, the dentifrices containing 1100 μg F/g led to a significantly higher incorporation of F in nails compared with the low-F acidified dentifrice (750 μg F/g), showing a tendency of low-F dentifrice to reduce fluoride exposure by a cumulative effect.

Considering the cutoff point of 2 μg F/g in nails for the development of fluorosis[19], a very high number of individuals are at risk of developing some mild fluorosis, based on the present study, even in a non-fluoridated area. It is important to emphasize that the *tutti-frutti*-flavored toothpaste, which was highly praised by most children, may have enticed children to use a large amount of toothpaste, even though it was recommended that only one drop be used. This is confirmed by data from previous studies,[28] in which the flavor of the dentifrice contributed significantly to its greater intake.

The fluoride concentration in the children's nails was 15% lower in those who used dentifrice with 750 μg F/g, than in those who used dentifrice with 1,100 μg F/g. This fact is very interesting because the 30% reduction of fluoride concentration in the dentifrice resulted in 15% less incorporation of fluoride in nails. Compared with the study[17] performed in the city of Bauru-SP, an optimally fluoridated area, using dentifrices with 550 μg F/g x 1,100 μg F/g, a 50% reduction in fluoride concentration in dentifrice resulted in a 30% reduction of F incorporation in nails (2.57 μg F/g x 1.80 μg F/g). Despite the limitations of the present study and taking into account previous studies, it can be concluded that nails are suitable biomarkers to identify groups of children exposed to normal or pediatric dentifrices and consequently at higher risk of developing dental fluorosis.

Conclusion

In spite of the high fluoride exposure observed in all the groups studied, it can be concluded that a lower F concentration in the dentifrice is a relevant factor in reducing the excessive intake of this ion, even in a non-fluoridated area. Therefore, the use of a low-F acidified dentifrice (750 μg F/g) is a promising alternative, especially for children whose parents still choose non-fluoridated dentifrices. In addition, the greater incorporation and maintenance of F in the biofilm by the low-F acidic dentifrice is very important, as this may reflect its potential anti-caries effect.

Compliance with ethical standards

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The full trial protocol can be accessed at http://clinicaltrials.gov/ (NCT03761485).

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