COHORT STUDY

Fluoride Varnish Applications in Preschoolers and Dental Fluorosis in Permanent Incisors: Results of a Nested-cohort Study Within a Clinical Trial

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Abstract: ***Purpose:*** *To compare the prevalence and severity of fluorosis in the permanent maxillary incisors of children who had participated in a two-year randomized placebo-controlled clinical trial on fluoride varnish application in the primary dentition and to assess children's esthetic perception of their teeth.* ***Methods:*** *Parents of 200 one- to four-year-old children who had received biannual applications of fluoride or placebo varnish were contacted four years after the end of the trial. Two calibrated examiners assessed dental fluorosis using the Thylstrup and Fejerskov index (TF) and interviewed the children regarding their perceptions of teeth appearance.* ***Results:*** *Fluorosis (TF equals at least one) and esthetically objectionable fluorosis (TF equals at least three) were observed in 38 (30.9 percent) and eight (6.5 percent) children, respectively. There was no statistically significant difference in fluorosis prevalence between children who had received fluoride or placebo varnish. Children's responses regarding the esthetic perceptions of their teeth showed no statistically significant difference between children with and without fluorosis.* ***Conclusions:*** *Fluoride varnish applications in preschoolers were not associated with any level of fluorosis in their permanent maxillary incisors. The fluorosis found in this study did not influence the children's esthetic perception of their teeth. (Pediatr Dent 2016;38(5):414-8) Received March 14, 2016 / Last Revision June 26, 2016 / Accepted June 28, 2016*

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Fluoride (**F**) varnish is one of the various effective methods of F delivery in the control of dental caries.[1] The rationale behind its development was to prolong the contact time between F and dental enamel, allowing it to act as a slow-releasing reservoir of loosely bound and tightly bound F.[2] Despite the high F concentration, acute toxicity is unlikely due to the small amount of varnish applied and rapid setting time, making it safe to apply in young children.[3] Moreover, plasma F levels after varnish application were found to be far below those considered toxic.[4] Regarding chronic toxicity, the only adverse effect to be expected would be the development of dental fluorosis. However, dental fluorosis related to F varnish applications could practically be ruled out, as these applications are sporadic, and dental fluorosis is caused by the chronic absorption of F that is ingested for long periods during tooth development.[5,6] However, although unlikely, the hypothesis that fluorosis is not related to professionally applied F has never been tested within an experimental design.

The exact mechanism by which F varnishes could cause fluorosis is unclear; similarly, however, the exact mechanism by which F varnishes can have a six-month anticaries effect from a single application is yet to be established.[7] Thus, one could hypothesize that, if varnishes slowly deliver sufficient F to the oral environment in order to have a measurable positive anticaries effect, they could also contribute to increased chronic F exposure and, therefore, the risk of fluorosis development, especially when used in very young children who are also exposed to fluoridated toothpaste and water.

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The purposes of this study were to: (1) compare the prevalence and severity of dental fluorosis in the permanent maxillary incisors of children four years after a two-year randomized placebo-controlled clinical trial on biannual F varnish application in the primary dentition; and (2) assess children's esthetic perception of their teeth.

Methods

This study was approved by the Research Ethics Committee of the Pedro Ernesto Hospital at the Rio de Janeiro State University, Brazil, and all parents read and signed informed consent forms. This was a nested-cohort study within a randomized controlled trial. Study participants had previously taken part in a randomized placebo-controlled clinical trial about the effectiveness of biannual F varnish application on caries incidence in the primary dentition. Two-hundred one- to four-year-olds had been randomly allocated to either the test group (biannual F varnish application) or control group (biannual placebo varnish application) and were followed for two years. Details of sample size calculation, randomization, and recruitment can be found elsewhere.[8] The parents of all 200 children were contacted by telephone or letter four years after the end of the clinical trial in order to make an appointment.

Two examiners participated in a theoretical training, which was carried out using the *Handbook of Fluorosis for Health Workers*[9] and the original paper that proposes the Thylstrup and Fejerskov fluorosis index.[10] The criteria described by Russel were also studied to differentiate mild forms of fluorosis from nonfluoride enamel opacities.[11] Next, pictures containing images of different stages of fluorosis in the permanent maxillary incisors were evaluated. Clinical training included the examination of 23 eight- to 11-year-olds presenting teeth with different stages of fluorosis. After each examination, the two examiners discussed the results, and any disagreement was solved by

consensus. Finally, 27 eight- to 11-year-olds were independently examined to assess the interexaminer reproducibility. None of these children took part in the F varnish trial.

Dental examinations were performed by two examiners who had not participated in the clinical trial and were blind regarding the intervention each child had received. All children were examined in a dental office using a dental mirror, under natural light, after supervised toothbrushing. The buccal surfaces of the permanent maxillary incisors were classified for fluorosis after being dried with cotton rolls. Once fluorosis was diagnosed, the child received scores for the two most severely affected teeth. Whenever these two teeth presented different scores, classification was determined by the highest score.[9]

Information regarding gender, age, socioeconomic status (**SES**), children's exposure to F toothpaste and fluoridated water, and toothbrushing habits were collected. All children answered the Brazilian version[12] of a questionnaire that measures concerns caused by the children's and their parents' perceptions of dental appearance.[13] In this study, we analyzed a question on distress ("During the past two months, how upset have you been about the way your teeth look?") and a question regarding social concern ("During the past two months, how much has the way your teeth look kept you from smiling freely?"). Both questions were graded using a Likert-like scale with the following levels: a lot; a little; very little; not at all; and don't know. A third question asked about the children's self-perceived teeth discoloration ("Please rate your teeth according to the following: very white; white; not white or stained; slightly stained; and very stained"). The last question asked children about their opinion using a statement on how pleasant their teeth color was ("The color of my teeth is pleasing and looks nice"), with the following response options: strongly agree; agree; neither agree nor disagree; disagree; and strongly disagree.

Data were entered in MS Excel™ (Microsoft Corp., Redmond, Wash., USA), and analyzed in Stata 11.1® software (Stata-Corp, College Station, Texas, USA). Interexaminer reproducibility was calculated using the quadratic weighted kappa coefficient. The difference between fluorosis prevalence in the test and control groups was analyzed using Fisher's exact test. Children's perceptions of teeth appearance were also analyzed using Fisher's exact test. The significance level was set at five percent. Post hoc secondary analysis was conducted to assess the effect of F varnish application according to children's age at baseline.

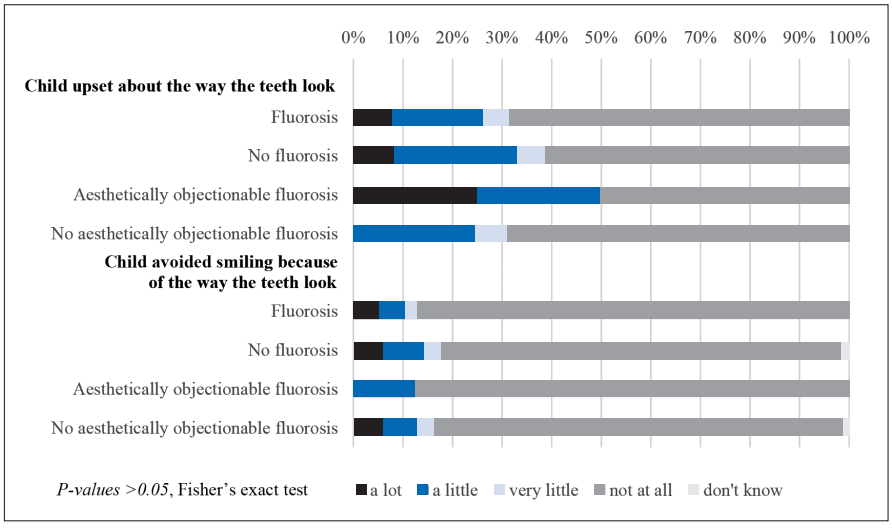

Figure 1. Frequency of children reporting distress and smile avoidance, because of teeth appearance, by presence and severity of fluorosis.

Results

The clinical trial enrollment took place from July 2006 to July 2007, and follow-up ended in September 2009. All parents of the 200 children who were randomized were contacted by telephone or letter, and appointments were scheduled between June and October 2013. A total of 123 (61.5 percent) children were re-examined: 63 originally belonged to the test group, and 60 belonged to the control group. Children who were examined for dental fluorosis at follow-up were similar to children who were enrolled in the F varnish trial regarding gender, SES, age, use of F toothpaste, and dental caries status (Table 1).

The age of the children examined for dental fluorosis varied from seven to 11 years old (mean equals 9.4±0.9 [SD]), and most families (93 percent) earned between $255 and $599 (U.S. dollars) per month. All families used 1,000 ppm F toothpaste, and 39 percent reported that they started using it before the first year of life. However, in the follow-up study, 39 percent brushed twice a day, 30.9 percent brushed once a day, and 30.1 percent brushed three times a day. At the outset of the F varnish trial, 79.5 percent of the children used F toothpaste, and 96.5 percent had access to optimally fluoridated water.[8]

The interexaminer reproducibility for fluorosis, measured by the quadratic weighted kappa coefficient, was considered substantial (k equals 0.73) according to Landis and Koch.[14] Thirty-eight children (30.9 percent) had some form of dental fluorosis. The most frequent scores were TF 2 (18 children) and TF 1 (12 children). TF 3 was found in seven children, and TF 5 was found in one child. When only the cases of esthetically objectionable fluorosis were considered (TF equals at least 3), the prevalence of fluorosis decreased to 6.5 percent.

Table 2 shows that there was no statistically significant difference between fluorosis in the permanent maxillary incisors among the children who had received biannual F varnish applications and those who had received biannual placebo varnish applications (P=0.44). When only esthetically objectionable fluorosis was considered, the difference remained statistically nonsignificant (P=0.48).

When children were asked whether they were upset or avoided smiling because of teeth appearance, there was no statistically significant difference among those who had some level of fluorosis or no fluorosis at all (P>0.05). The same was observed when children were asked whether the color of their teeth was pleasing and looked nice. Children who had esthetically objectionable dental fluorosis tended to state that their teeth were stained more often than those who did not; yet this difference was not statistically significant (P=0.06; Figures 1-3).

Discussion

Due to ethical concerns, it is not acceptable to design a trial to assess harmful interventions. Thus, one should not expect there to be a trial

Table 1. CHARACTERISTICS OF THE PARTICIPANTS IN FLUORIDE VARNISH (FV) AND PLACEBO (PV) GROUPS AT BASELINE*

Characteristic	Participants randomized to FV and PV						Participants in the FV and PV groups who remained in the study and were examined 4 years after the end of the randomized controlled trial					
	FV group (N=100)		PV group (N=100)		All children (N=200)		FV group (N= 63)		PV group (N=60)		Children lost to follow-up (N=77)	
Gender† N (%)												
Female	50	(50)	43	(43)	93	(47)	31	(49.2)	28	(46.7)	34	(44.2)
Male	50	(50)	57	57	107	(53)	32	(50.8)	32	(53.3)	43	(55.8)
Socioeconomic status† N (%)												
B	9	(9)	4	(4)	13	(6.5)	6	(9.5)	2	(3.3)	6	(7.8)
C	57	(57)	65	(65)	122	(61.0)	36	(57.1)	41	(68.3)	44	(57.1)
D or E	33	(33)	29	(29)	62	(31.0)	21	(33.4)	16	(26.7)	26	(33.4)
Not provided	1	(1)	2	(2)	3	(1.5)	0	(0)	1	(1.7)	1	(1.3)
Age‡												
Mean±(SD) ys	2.5	(0.8)	2.3	(0.9)	2.4	(0.9)	2.6	(0.9)	2.3	(0.9)	2.3	(0.8)
Use of fluoride toothpaste† N (%)												
Yes	84	(84)	75	(75)	159	(79.5)	52	(82.5)	44	(73.3)	63	(81.8)
No	15	(15)	23	(23)	38	(19)	11	(17.5)	16	(26.7)	11	(14.3)
Not informed	1	(1)	2	(2)	3	(1.5)	0	(0)	0	(0)	3	(3.9)
Dental caries status												
$d_3$mfs‡ mean±(SD)	0.6	(1.6)	1.0	(2.1)	0.8	(1.9)	0.4	(1.2)	1.0	(2.2)	1.0	(2.0)
Children with dentin caries§ N (%)	21	(21)	26	(26)	47	(24)	10	(15.9)	15	(25.0)	22	(28.6)

* All *P* values were >0.05; socioeconomic status was measured using the Brazilian Economic Classification Criteria, which categorizes people into five socioeconomic categories (A and B=high socioeconomic status, C=medium socioeconomic status, D and E=low socioeconomic status); $d_3$mfs=number of cavitated decayed (at dentin level), filled and extracted dental surfaces in primary teeth.

† Univariate analyses were performed using chi-square test.

‡ Univariate analyses were performed using t test.

§ Univariate analyses were performed using two-sample test of proportion.

designed to specifically assess the occurrence of dental fluorosis per se. However, there is still room to design clinical trials to assess the benefits of different methods of F delivery, and researchers should not miss the opportunity to investigate the adverse effects related to F exposure. In fact, the Cochrane systematic review on the effects of topical F on dental fluorosis suggested that trials assessing the effectiveness of different types of topical F (including toothpastes, gels, varnishes, and mouthrinses) should include an adequate follow-up period in order to collect data on fluorosis.[15]

To assess an adverse long-term effect such as dental fluorosis, trials must account for a long follow-up period. Moreover, to be able to verify whether the intervention of interest (F application) has the potential to cause fluorosis in the permanent maxillary incisors, the intervention must occur at a very early age (first four years of life),[6] whereas the assessment of the outcome (dental fluorosis) must be at eight to nine years old when these teeth are sufficiently erupted. It becomes clear that such a trial is difficult to conduct. To the best of our knowledge, this study presents, for the first time, a follow-up of a F varnish trial in the primary dentition that assessed the occurrence of dental fluorosis in the permanent maxillary incisors. There are only two other studies that also assessed the prevalence of dental fluorosis after the end of randomized trials that aimed primarily to assess the anticaries effect of F. However, these two trials tested the effectiveness of F toothpastes and

Table 2. DISTRIBUTION OF THE CHILDREN WITH AND WITHOUT FLUOROSIS ACCORDING TO THE INTERVENTION GROUPS (N=123)

Fluorosis	Yes N (%)	No N (%)	*P*-value*
Fluoride varnish	17 (27.0)	46 (73.0)	
Placebo varnish	21 (35.0)	39 (65.0)	0.44
Total	**38 (30.9)**	**85 (69.1)**	
Esthetically objectionable fluorosis			
Fluoride varnish	3 (4.8)	60 (95.2)	
Placebo varnish	5 (8.3)	55 (91.7)	0.48
Total	**8 (6.5)**	**115 (93.5)**	

* Fisher's exact test.

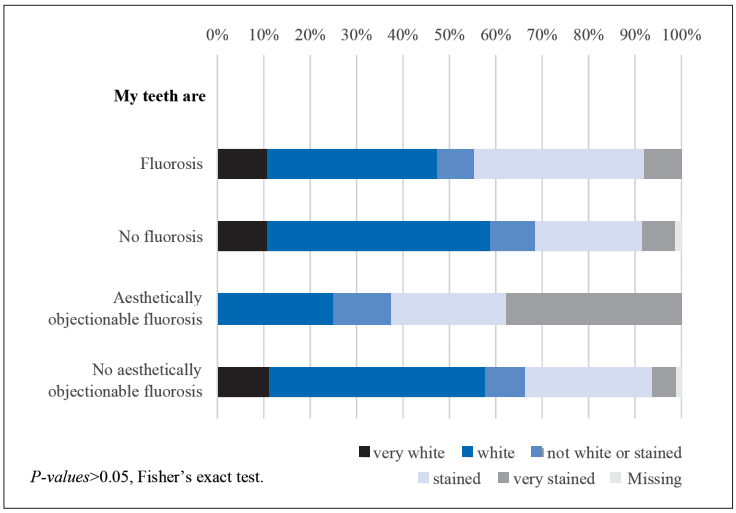

Figure 2. Self-ratings of tooth discoloration by presence and severity of dental fluorosis.

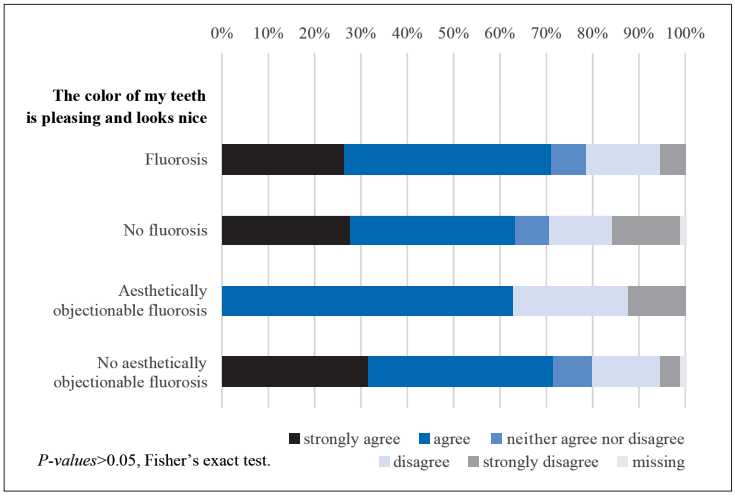

Figure 3. Self-ratings of satisfaction with tooth color by presence and severity of dental fluorosis.

not of professionally applied F.[16,17] Their pooled results show that the use of standard F toothpaste, compared to low F toothpaste, increases the risk of all types of fluorosis[15] but does not increase the risk of esthetically objectionable fluorosis.[18] To date, our study, together with these two other trials, comprises the only clinical experi-mental evidence of the effects of topical F on the occurrence of fluorosis.

We found a prevalence of all fluorosis levels of approximately 31 percent, which is below what is expected (40 to 47 percent) when F levels in the water range from 0.7 to one ppm.[19] In fact, two studies carried out in other Brazilian cities with optimally fluoridated water showed higher prevalences of fluorosis: 59 percent[20] and 72 percent.[21] The extent to which the prevalence of fluorosis described in this study could, in fact, be regarded as incidence of fluorosis is debatable. Because of the biological nature of fluorosis, it is not feasible to design a trial that will assess teeth that were fluorosis-free in the baseline and where, after a follow-up period, some would have developed fluorosis. Thus, since the development of fluorosis occurs before teeth erupt but can only be assessed after they erupt, any attempt to assess the incidence of fluorosis in a clinical trial would be similar to the one employed in our study.

On the other hand, it has been shown that long exposure time (more than two of the first four years of life) increased the risk for developing dental fluorosis in the permanent maxillary incisors.[6] As children's ages in the baseline varied from one to four years old, it could be possible that fluorosed teeth detected in the older children would not be related to the intervention (i.e., F varnish application) but rather to other F exposures that happened earlier in their lives. However, we found no statistically significant difference in terms of mean age at baseline between children who developed fluorosis and those who did not. Moreover, our post hoc analysis did not detect any difference in the occurrence of fluorosis or esthetically objectionable fluorosis when the sample was stratified according to the children's age at baseline; in other words, children equal to or younger than two years old at the outset of the trial presented the same level of fluorosis when compared to children who were older than two years of age when enrolled in the trial. These findings reinforce the assumption that our results of fluorosis prevalence could be interpreted as fluorosis incidence.

No statistically significant differences were found in fluorosis between children who had received biannual F varnish application or biannual placebo varnish application, even when only cases of esthetically objectionable fluorosis were considered. Actually, more cases of fluorosis and esthetically objectionable fluorosis were detected in the placebo group. This suggests that F varnish application, in addition to F in the water and in the toothpaste, does not increase the risk of a child developing dental fluorosis.

The presence of fluorosis did not appear to influence children's responses to questions related to teeth appearance. Some children answered that they were upset or avoided smiling because of teeth appearance; however, that was not associated with fluorosis, irrespective of its level. Also, some children with no fluorosis at all disagreed that the color of their teeth was pleasing and looked nice. This emphasizes that other conditions, such as dental caries and dental trauma, may be affecting children's perceptions of their teeth appearance, as shown in previous studies.[21-23] As might be expected, teeth were more often regarded as being stained or very stained in children with esthetically objectionable fluorosis, although no statistically significant difference was found.

It should be noted that the F varnish trial was designed to assess the incidence of dental caries, not the incidence of fluorosis, which means that the sample size calculation did not take both outcomes into account. The occurrence of esthetically objectionable dental fluorosis is very uncommon. Considering the prevalence observed in this study for test and control groups, a trial would need to enroll approximately 1,000 children in each group in order to have a power of 80 percent to detect a statistically significant difference in the percentages of fluorosis in both groups, if such a difference actually existed. Thus, the evidence provided in this study cannot rule out the effect of F varnish application on the development of fluorosis.

Conclusions

Based on this study's results, the following conclusions can be made:

1. Biannual F varnish applications in preschoolers were not associated with the occurrence of any level of fluorosis in permanent maxillary incisors.
2. Children's responses to questions regarding teeth appearance did not differ among those with any level of fluorosis and those with no fluorosis at all.

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