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Caries-preventive effectiveness of fluoride varnish as adjunct to oral health promotion and supervised tooth brushing in preschool children: A double-blind randomized controlled trial

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ABSTRACT

Objectives: To evaluate the effect of biannual fluoride varnish applications in preschool children as an adjunct to school-based oral health promotion and supervised tooth brushing with 1000 ppm fluoride toothpaste.

Methods: 424 preschool children, 2–5 year of age, from 10 different pre schools in Athens were invited to this double-blind randomized controlled trial and 328 children completed the 2-year programme. All children received oral health education with hygiene instructions twice yearly and attended supervised tooth brushing once daily. The test group was treated with fluoride varnish (0.9% diflurosilane) biannually while the control group had placebo applications. The primary endpoints were caries prevalence and increment; secondary outcomes were gingival health, mutans streptococci growth and salivary buffer capacity.

Results: The groups were balanced at baseline and no significant differences in caries prevalence or increment were displayed between the groups after 1 and 2 years, respectively. There was a reduced number of new pre-cavitated enamel lesions during the second year of the study ($p = 0.05$) but the decrease was not statistically significant. The secondary endpoints were unaffected by the varnish treatments.

Conclusions: Under the present conditions, biannual fluoride varnish applications in preschool children did not show significant caries-preventive benefits when provided as an adjunct to school-based supervised tooth brushing with 1000 ppm fluoride toothpaste.

Clinical significance: In community based, caries prevention programmes, for high caries risk preschool children, a fluoride varnish may add little to caries prevention, when 1000 ppm fluoride toothpaste is used daily.

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1. Introduction

Caries in the primary dentition is a common public health problem that may affect the quality of life of children as well as their families.[1,2] The condition affects a large number of children, and a clear socioeconomic gradient is evident,[3,4] underlining the need for targeted oral health programmes directed to vulnerable populations or groups of children.[5,6] Daily plaque removal together with topical fluoride application is the basis of any preventive programme for dental caries. Recent systematic reviews have established strong evidence for daily use of fluoride toothpaste as the most cost-effective self-applied method of preventing caries in preschool children.[7–9] In addition, an increased prevented fraction is evident when the tooth brushing is supervised by an adult.[10] For preschool children with increased caries risk, regular fluoride varnish applications are often advocated as an effective, safe and practical way to increase fluoride exposure in the oral biofilm.[11–13] Fluoride varnish, in conjunction to caregiver oral health counselling has been shown to prevent dental caries in this age group, both in public health centers[14] and large community based preventive programmes.[15] To our knowledge, few studies have combined supervised use of 1000 ppm fluoride toothpaste with fluoride varnish applications for early childhood caries prevention. The purpose of this study was, therefore, to evaluate the effect of biannual fluoride varnish applications in preschool children as an added measure to a preventive programme consisting of school-based oral health education and supervised tooth brushing with 1000 ppm fluoride toothpaste. The null hypothesis was that the caries incidence over a 2-year period would not differ from a group treated with placebo varnish.

2. Materials and methods

2.1. Study group

Children from 10 public preschools, located in medium and low socioeconomic areas of Athens, Greece, were invited to participate. In Greece, there is no free of charge dental treatment even for young children. Furthermore there are not any organized preventive programmes for preschool children and regular dental care is provided only in private settings. Emergencies can be treated in the children's hospital free of charge, but no further treatment will be provided. The schools for this study were selected based on previously established caries risk profiles[16] and the schools with the highest risk were considered for enrollment. The total number of the preschools in Athens was 90 and they were located in areas of all socioeconomic levels. A cluster sampling technique was used to draw a sample representative of the preschool age population in this area.[16] For the present study, the sample size was calculated and then ten schools were chosen that had the appropriate number of children and were of high risk. The high risk children were chosen because it was anticipated that they would benefit more than low risk individuals from the preventive interventions. Children can attend preschools until they have completed their sixth year of age so they can enrol in the first grade of primary school. The parents received an invitation letter explaining the purpose of the study and for signing their informed consent. The inclusion criteria were: (i) being between 2 and 5 years of age and attending one of the preselected public preschools, (ii) born in Greece, and (iii) residing in the greater area of Athens. Children born outside of Greece and children taking antibiotics within the last 2 weeks prior to the clinical examination were excluded. In total, 424 children were eligible, 409 were enrolled and randomized, and 328 completed the programme. A flow-chart with main reasons for the attrition is presented in Fig. 1.

2.2. Study design

The project employed a double-blind randomized controlled trial with two parallel arms conducted between 2009 and 2011. The duration was two years. The protocol was approved by the Ethics Committee of the Dental School at the University of Athens, Greece.

2.3. Randomization

The children were stratified with respect to caries risk before the randomization with the objective to have similar number of high and low risk caries children in each group. A child was considered at high risk if there was at least one carious lesion (pre-cavitated or cavitated) and/or presence of mutans streptococci in the microbiological test. Random permuted blocks of size 8 were used and the randomization lists were produced with computer software (http://mahmoodsaghaei.tripod.com/Softwares/randalloc.html). The random lists were generated by the principal investigator and a secretary allocated the names of the children for every school. An assistant, not participating in the field study, prepared the bottles with the varnish according to the allocation lists, placing a tag for each subject. The allocation of the subjects was unknown to the examiner, parents and their children and not unveiled until after the statistical calculations. Allocation to treatment groups was made approximately one week after the baseline examination, in order to calculate the caries risk based on examination data.

2.4. Questionnaire

All parents that agreed to participate completed a questionnaire on demographic data and general health. In case the parents were of foreign origin, the teachers assisted them with the questionnaire.

2.5. Oral health education and daily tooth brushing

The teachers and headmasters of the participating schools were informed about the study protocol and were requested to actively support the oral health promotion programme. All children and their teachers received comprehensive oral health education in the class room by dental professionals, including oral hygiene instructions and dietary advice, twice a year, over the two-year period. The information was adapted to match the age of the children. Furthermore, all children received a toothbrush to use at school. A pea-size amount of

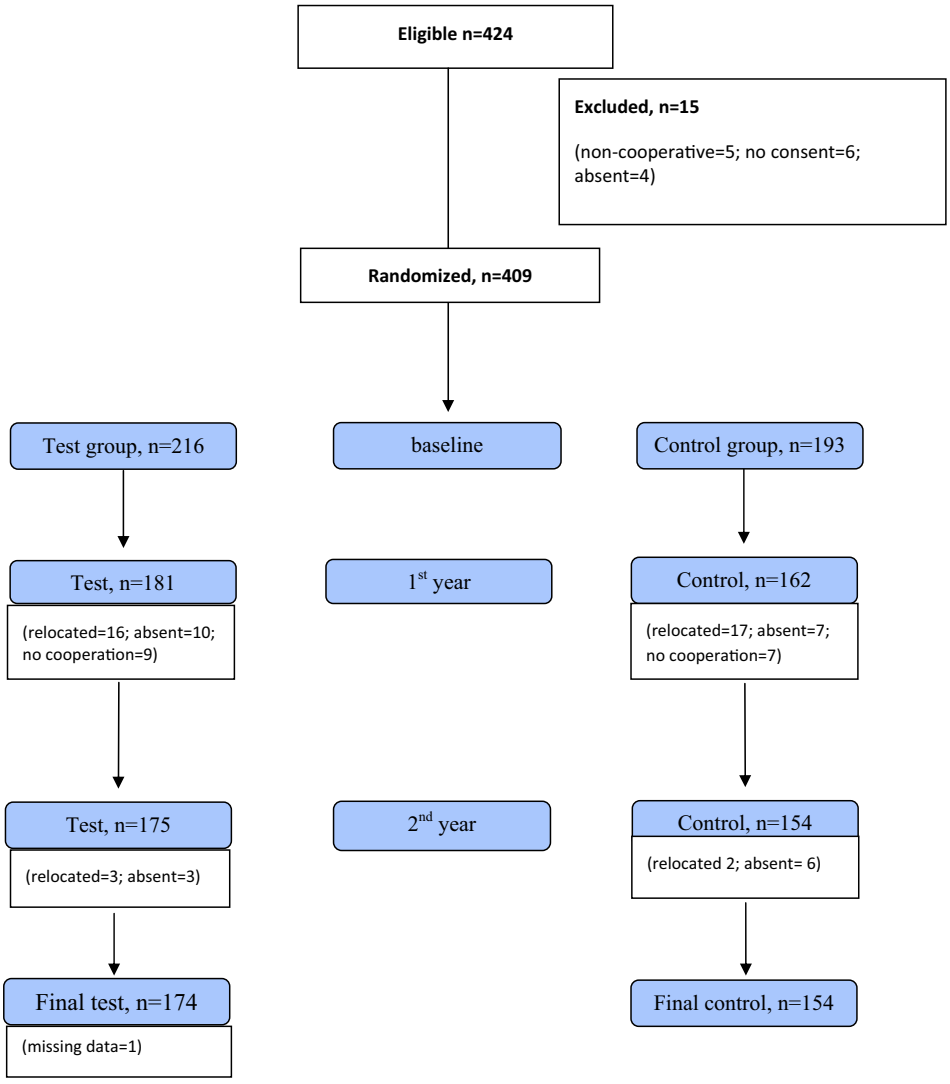

Fig. 1 – Flow-chart from baseline to the 2nd year with main reasons for dropping out.

1000 ppm fluoride toothpaste was applied on the brush by the teacher who supervised and assisted the brushing once a day (after breakfast or after lunch). The parents were instructed by the teachers to brush their children's teeth at night and a leaflet with oral hygiene instructions was provided.

2.6. *Study groups*

Children allocated to the test group received fluoride varnish (0.9% diflurosilane) applications twice a year (Fluor Protector®, Ivoclar Vivadent AG, Schaan, Liechtenstein). The applications were performed at 6 months intervals in the schools by one single dentist. The teeth were cleaned and dried with gauze and approximately 0.2 ml of the varnish was applied with a micro-brush on all maxillary and mandibular teeth. Setting was allowed for 2 min. The children of the control group received biannual applications of a placebo varnish without fluoride. Both varnishes (test and placebo) had the same smell, texture and packed in identical boxes. The parents were instructed not to brush the day of the varnish application. During the study, parents were also informed about the need for restorative treatment for those children exhibiting cavities.

2.7. *Clinical examinations*

All children were clinically examined at inclusion (baseline) and at the end of the first and second years. The examinations were performed in the classroom by one single examiner, using a penlight as light source, a dental mirror and a periodontal explorer. The primary outcome measures were caries prevalence and caries increment. Secondary outcomes were visible plaque index (VPI), gingival bleeding index (GBI), mutans streptococci (MS) growth and saliva buffer capacity (SBC).

Dental caries was recorded at cavity level according to WHO criteria[17] and expressed as dmfs. Missing teeth were recorded only if there was information that they were extracted due to caries and very few children had restorations. The number of surfaces with early enamel caries (ec) lesions (chalky white spots with no cavitation) was registered

separately. No radiographs were exposed. Dental plaque (VPI) was scored as ''presence'' or ''absence'' and expressed as the ratio of surfaces covered with plaque in relation to the total number of surfaces.[18] Similarly, the gingival condition (GBI) was expressed as the number of bleeding sites after gentle probing in relation to the total number of accessible sites. The caries increment (Δdmfs, Δec) was computed for each individual by counting the number of surfaces that changed from sound to decayed, filled or missed over the study period. In the case of exfoliated anterior incisors, the teeth with caries experience (cavitated or early enamel lesions) at baseline were considered as no change in caries increment at the follow-ups. No caries reversals were identified.

2.8. Salivary tests

The presence of mutans streptococci (MS) was estimated with the CRT® bacteria (Ivoclar Vivadent AG, Schaan, Principality of Liechtenstein) according to the method suggested by Twetman & Grindefjord.[19] The tubes were then incubated for 72 hours in 37 °C and scored in two categories, ''growth'' or ''no detectable growth''. Saliva buffer capacity (SBC) was evaluated using CRT® buffer (Ivoclar Vivadent AG, Schaan, Principality of Liechtenstein) and the results were divided into three categories; ''low'', ''medium'' and ''high'' buffer capacity.

2.9. Intra-examiner reliability

A single examiner performed all clinical examinations and evaluated the saliva tests. The intra-examiner reliability was tested as follows: (a) 20 children were examined and dental caries was recorded. A second examination of the same children was performed 15 days later, (b) 30 strip mutans tests were evaluated and then stored in 4 °C. After 15 days, the strips were rescored, and, (c) 30 buffer strips were photographed and the photos were re-evaluated after 15 days. The intra-examiner reliability was $k = 0.89$ for dental caries, $k = 0.82$ for the MS test and $k = 0.87$ for the SBC test.

2.10. Statistical analysis

All data were processed by the Stata 13.1 software (Stata Corp, College Station, TX, USA). Descriptive statistics were calculated for the two groups. Primary and secondary outcome measures were compared between groups for the first and second year examinations. The comparisons between the two groups were tested using independent sample t test for continuous variables and χ^2 test for categorical variables. In the cases where the distribution of the data was not normal, non-parametric tests were also used to confirm the comparison. They all gave the same results, hence only the parametric ones are presented. Statistical significance was set at $p = 0.05$.

3. Results

3.1. Baseline data and drop-outs

The baseline characteristics for the experimental groups, as well as the dropouts, are presented in Table 1. The test and the control groups were balanced in all aspects, including socio-demographic data and general health. The dropout rate was 19.8% over the 2-year study. There was no statistically significant difference between the dropouts and those that remained in the study, with the exception of a minor difference in age ($p < 0.05$). All children received four fluoride applications except for 17 children that received three applications, 10 in control group and 7 in test group.

Table 1 – Baseline characteristics (mean and standard deviation, or percent) of selected key variables. No statistically significant differences were found between the test and control groups.

Variable	Group		
	Test $n = 216$	Control $n = 193$	Drop outs $n = 80$
Sex (boys, %)	49.5	51.3	53.9
Age, year	3.4 (0.8)	3.4 (0.8)	3.6 (0.8)[a]
dmfs	3.1 (7.1)	2.5 (5.6)	3.5 (7.5)
VPI	0.4 (0.3)	0.5 (0.3)	0.4 (0.3)
GBI	0.1 (0.1)	0.1 (0.1)	0.1 (0.1)
MS (prevalence, %)	35.4	31.0	23.7
SBC (low, %)	17.0	15.2	19.2

[a] The mean age of the drop-outs were significantly higher compared with those that completed the programme ($n = 328$, mean age 3.3 (0.8) year; $p < 0.05$).

3.2. Caries prevalence and incidence

Caries data on those that completed the trial are presented in Tables 2–4. There were no significant differences in caries prevalence between the test and the control group, neither at baseline nor at the follow-ups (Table 2). The cumulative dmfs-index and detailed information on the number of decayed,

Table 2 – Caries prevalence (dmfs > 0) expressed as percent at baseline and after first and second year.

Group	n	Baseline	1st year	2nd year
Test	174	37.5	63.0	64.8
Control	154	37.8	64.8	65.8

Table 3 – Mean (SD) dmfs index and descriptive information (mean, SD) regarding the components decayed (ds), missed (ms) and filled (fs) surfaces, as well as the number of pre-cavitated enamel caries lesions (ec), in the test and control groups at each examination.

Group	Variable	Baseline	1st year	2nd year
Test	dmfs	3.1 (7.3)	5.2 (9.2)	5.8 (9.5)
	ds	3.1 (7.1)	4.8 (8.5)	4.9 (8.0)
	ms	0 (0)	0.1 (1.9)	0.2 (2.3)
	fs	0 (0.1)	0.3 (2.1)	0.6 (2.5)
	ec	0.6 (1.5)	1.0 (1.1)	1.2 (1.1)
Control	dmfs	2.5 (5.6)	4.9 (8.0)	5.5 (8.8)
	ds	2.3 (5.2)	4.6 (7.6)	5.3 (8.3)
	ms	0 (0)	0.1 (1.0)	0.1 (1.0)
	fs	0.2 (1.3)	0.2 (0.9)	0.3 (1.1)
	ec	0.5 (1.2)	1.1 (2.1)	1.4 (2.6)

Table 4 – Mean (SD) caries increment (Δdmfs, Δec) in the test and control groups during the first and second year of the study.

Index and time	Test group $n = 174$	Control group $n = 154$	p
Δdmfs, baseline – 1st year	2.1 (4.5)	2.3 (4.7)	0.75
Δdmfs, 1st year–2nd year	0.8 (2.2)	1.1 (2.3)	0.28
Δdmfs, total	2.9 (5.3)	3.0 (5.2)	0.82
Δec, baseline – 1st year	0.3 (1.5)	0.6 (2.0)	0.20
Δec, 1st year–2nd year	0.2 (1.4)	0.5 (2.5)	0.05
Δec, total	0.6 (1.9)	0.9 (1.7)	0.12

missed and filled surfaces at each examination is shown in Table 3. The d-component dominated in both groups and few fillings and teeth missed due to caries were registered. There were no significant differences between the groups. The computed caries increment is summarized in Table 4. Interestingly, the caries increment was higher during the first year in both groups; the mean dmfs increment did not differ significantly between the two at any time point. The number of surfaces with pre-cavitated enamel caries (ec) did not differ between the treatment groups at baseline. The increment was high in both groups during the first year of the study but significantly lower in the test group during the second year ($p = 0.05$).

3.3. *Secondary endpoints*

Secondary outcome measures (VPI, GBI, MS, SBC) at each examination, are presented in Table 5. The fluoride varnish intervention did not affect the secondary outcome measures in a significant way at any time point.

3.4. *Harm*

No serious adverse effects following the varnish applications were noted. In some cases, the smell of the varnish was unpleasant to the younger children, but the problem was overcome using appropriate behaviour management.

4. Discussion

In this randomized clinical trial, a preventive programme for preschool children, including oral health promotion, dietary education, daily supervised school-based tooth brushing and biannual fluoride varnish applications was evaluated in preschool children. Although the children were recruited from schools located in high caries risk areas,[16] further individual risk stratification (high vs. low) was employed before randomization. The main finding was that the fluoride varnish applications tended to reduce the incidence of manifest caries and early enamel lesions, but not significantly different from supervised brushing alone. However, the pattern effect sizes (i.e., differences in point estimates) followed the same direction for each of the endpoints and at each time period which indicated a weak protective effect in favour of the active varnish. No effect on caries prevalence or the number of cavitated lesions was displayed. Thus, the null hypothesis could not be rejected. Our result was in contrast with most previous trials in which prevented fractions between 5% and 53% following fluoride varnish applications have been established.[11] On the other hand, Hardman and co-workers also failed to demonstrate a beneficial effect of twice-yearly fluoride varnish applications in young schoolchildren living in relatively deprived communities.[20] The explanation for the diverging results may be attributed to factors such as sample size, compliance, varnish composition and application frequency. First of all, the study was underpowered which makes any conclusions somewhat uncertain. A post-study power calculation indicated that a larger study group would have been needed to detect a 30% difference between the active and placebo varnishes with the present annual caries increment. Regarding compliance, the present study was planned in close cooperation with teachers and school authorities in order to ensure motivation and a smooth implementation of the project. The drop-out rate was less than 20% over the study period, the majority due to relocation, which was considered acceptable. The school setting encouraged compliance, participation in the daily oral health activities and facilitated the varnish applications. Practical problems in the different schools (e.g. issues with the brushing routine, time constraints, interference with the school daily schedule) were pragmatically solved through consensus between teachers and the dental professionals that carried out the oral health education.

In most studies concerning fluoride varnishes, a 5% NaF (22,600 ppm F) varnish has been applied [12] while in our study, a 0.9% difluorosilane (1000 ppm F) varnish was used. Although there are no head-to-head comparisons available in vivo, previous studies in vitro have shown equalities in fluoride uptake and retention in enamel between the different varnish formulas. An in vitro study has shown an increased retention of 0.9% difluorosilane varnish on the enamel surface in comparison to NaF varnish.[21] Furthermore, Seppä et al.[22]

Table 5 – Secondary outcome measures VPI, GBI, MS, SBC (mean and standard deviation, or percent) at each examination. No statistically significant differences were found between the test and control groups.

Variable	Group					
	Test			Control		
	Baseline	1st year	2nd year	Baseline	1st year	2nd year
VPI	0.44 (0.33)	0.35 (0.32)	0.22 (0.24)	0.46 (0.34)	0.34 (0.31)	0.24 (0.25)
GBI	0.07 (0.11)	0.10 (0.11)	0.10 (0.09)	0.07 (0.12)	0.11 (0.12)	0.11 (0.12)
MS (%)	21.8	35.4	29.7	25.4	31	27
SBC (low, %)	17	19.1	22.2	15.2	22.6	19.1

compared the concentration of fluoride that remained bound to the teeth after five semiannual applications using difluorosilane (Fluor-Protector) and NaF (Duraphat) varnishes and their results showed that the enamel on the teeth that received the Fluor-Protector had higher fluoride concentration and this remained after the last application and also two years later.[23] The difluorosilane varnish has also been shown to be effective in a clinical trial in preschool children.[24] Thus, there is some evidence that fluoride varnishes might not follow a classic dose–response relationship. There were no other potential anti-caries agents in the placebo varnish. Regarding the frequency of varnish applications, 2–4 times per year are considered as most cost-effective,[12] although a more frequent and intensive mode has been suggested.[25] It is possible that more frequent applications could have influenced our results but findings from proximal caries development in schoolchildren do not lend support to such a concept.[26] In spite of the fact that parents were informed on treatment needs, only 17 children, 11 in the test group and 6 in the control group, received dental care during the course of the study, which means that decisions on restorative care did not influence our results. A more likely explanation for our failure to demonstrate effectiveness was that the impact of fluoride varnish was obscured by the effectiveness of the daily supervised tooth brushing with 1000 ppm fluoride toothpaste, carried out in school during daytime and at home in the evenings. Similar observations have been made before [27] and calculations that fluoride varnish may generate a 10% elevated prevented fraction on top of fluoride toothpaste are based on studies in the young permanent dentition not including primary teeth.[28] In contrast to a similar community-based project in Australia,[29] both our experimental groups received comprehensive and regular oral health promotion throughout the study period, a fact that also may have contributed to the non-significant outcome. The tendency of a hampering effect on early enamel demineralization was not surprising and in agreement with findings that fluoride varnish primarily acts on surfaces that are sound at baseline.[11,30] In fact, a recent study in preschool children has shown that sound maxillary anterior surfaces received most caries-preventive benefit of fluoride varnish applications and significantly more than pits and fissures.[30] Although we do not present such site-specific results, the prevented effect was mainly evident during the second year of the trial, possibly indicating a "slowed-down" activity. In that aspect, an observation period longer than two years would have been helpful.

In conclusion, within the limitations of this study and under the present field conditions, biannual fluoride varnish applications in preschool children did not show significant caries-preventive benefits when provided as an adjunct to school-based supervised tooth brushing with 1000 ppm fluoride toothpaste. Further "real-life" studies are needed to identify cost-effective strategies to prevent early childhood caries in vulnerable populations.

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