

# Effect of a chlorhexidine/thymol and a fluoride varnish on caries development in erupting permanent molars: a comparative study

S. Flamee<sup>1</sup> · S. Gizani<sup>1</sup> · C. Caroni<sup>2</sup> · L. Papagiannoulis<sup>1</sup> · S. Twetman<sup>3</sup>

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## Abstract

**Aim** To compare the caries preventive effect of a chlorhexidine/thymol-containing antibacterial varnish with a fluoride varnish when topically applied during the eruption of permanent molars.

**Methods** The study group consisted of 189 patients, 5–14 years of age, with one 1st or 2nd permanent molar in the process of eruption. After stratification for type of molar and stage of eruption, the patients were randomised to either quarterly topical applications with an antibacterial varnish (Cervitec<sup>®</sup> Plus; CV group) or biannual applications with a fluoride varnish plus biannual treatments with placebo varnish (Fluor Protector; FV group). The duration of the study was 2 years. The primary endpoint was caries incidence (initial and cavitated) in the erupting molars and the secondary outcome was salivary mutans streptococci (MS) counts.

**Results** The groups were balanced with respect to socio-economy, oral hygiene, dietary habits and caries experience at baseline. The dropout rate was 11.6 %. The caries incidence was low (<10 %) in both groups and there was no significant difference between the CV and FV groups with respect to occlusal caries development in the erupting

molars (relative risk 1.08, 95 % CI 0.94–1.25). Significantly lower levels of salivary MS were disclosed in the CV group at the end of the study ( $p < 0.05$ ).

**Conclusions** No difference in occlusal caries development in young permanent molars was displayed after topical applications of either a chlorhexidine/thymol varnish or a fluoride varnish during tooth eruption.

**Keywords** Antibacterial agents · Children · Dental varnishes · Fissure caries · Mutans streptococci

## Introduction

It is well known that occlusal surfaces of permanent molars are highly susceptible to caries development during the first few years after tooth emergence (Korhonen et al. 2003, Mejàre et al. 2014). The vulnerability is mainly attributed to (1) a high plaque accumulation due to the fact that the complex fissures are partly covered by gingiva for a considerable period of time (Unkel et al. 1995), (2) lack of parental awareness on the tooth emergence (Araujo et al. 2002), and, (3) incomplete post-eruptive maturation of the enamel (Driessens et al. 1985). To combat the early fissure caries development, a number of topical interventions based on antibacterial agents and fluoride have been suggested until the occlusal surface can be fully protected by a fissure sealant (Twetman 2004; Hiiri et al. 2010; Tut and Milgrom 2010). The evidence for the use of chlorhexidine (CHX) varnishes is, however, still inconclusive (James et al. 2010) while a substantial caries-inhibiting effect has been reported from fluoride varnishes (Marinho et al. 2014). To our knowledge, there has been no direct comparison concerning the caries-preventive effect of CHX and fluoride varnishes on erupting molars available in the literature.

✉ S. Gizani  
stgizani@dent.uoa.gr

<sup>1</sup> Department of Paediatric Dentistry, Dental School, National and Kapodistrian University of Athens, 2 Thivon Str, Goudi, 11527 Athens, Greece

<sup>2</sup> Department of Mathematics, National Technical University of Athens, 9 Heron Polytechniou str, 15780, Zografou, Athens, Greece

<sup>3</sup> Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Nørre Allé 20, 2200 Copenhagen N, Denmark

The aim of the present study was therefore to evaluate the caries-preventive effect of chlorhexidine varnish when topically applied during the eruption period of permanent molars. The null hypothesis was that the effect would not differ from that of a fluoride varnish.

## Materials and methods

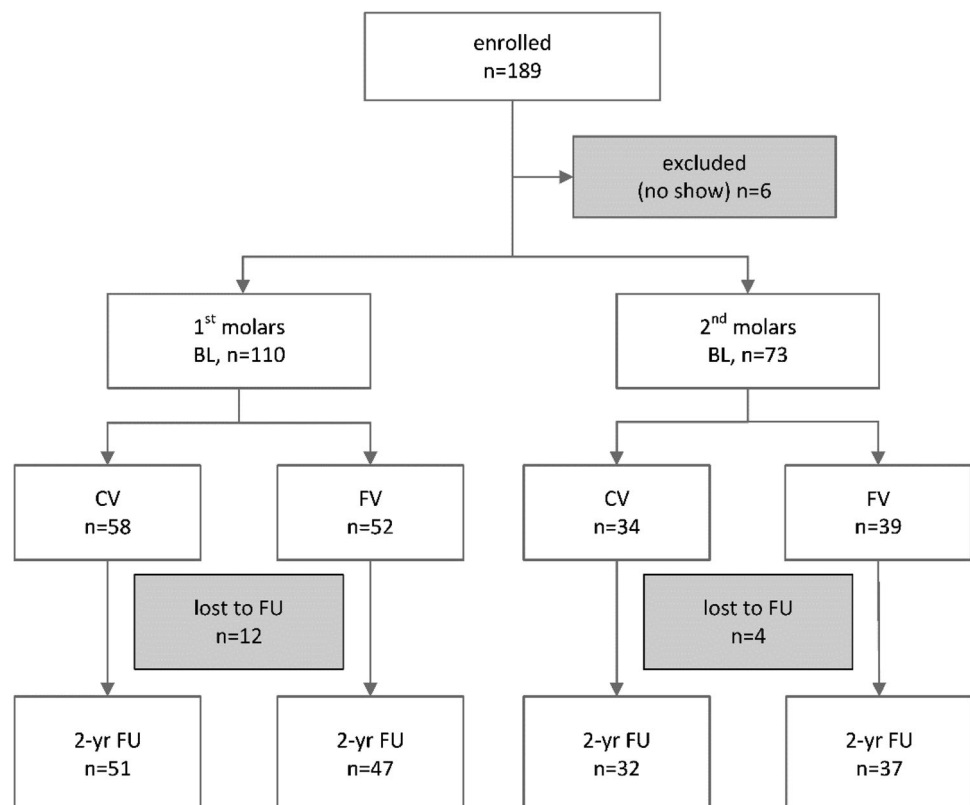
The study group consisted of 189 healthy children, 5–14 years of age, attending the Postgraduate Paediatric Dental Clinic, University of Athens, Greece. The inclusion criterion was one clinically sound, with no visible demineralisation or hypomineralisation, first or second permanent molar under eruption according to the index of Carvalho et al. (1991): Stage I = more than half of the occlusal surface covered by gingiva, Stage II = less than half of the occlusal surface covered by gingiva, and, Stage III = tooth partially erupted without gingival coverage. The main reason for subjects dropping out was moving away from the area of study, while six children that failed to attend at least three out of the five scheduled appointments per year were considered non-compliers and excluded. Children under treatment with systemic or local antibiotics during 2 weeks prior to baseline were excluded. A signed consent form was obtained from the parents after verbal and written information about the procedures and

potential side effects. A flowchart of the management of the participants is shown in Fig. 1. The total dropout rate was 11.6 % and the final material consisted of 167 patients, of which 98 had a first permanent molar under eruption and 69 the second molar under eruption. The baseline stage of molar eruption is presented in Table 1.

## Study design and intervention

The study employed a randomised single-blind design with two parallel arms. The duration of the study was 2 years after patient inclusion. The protocol was approved by the Ethics Committee of the Dental School, University of Athens, Greece. The primary endpoint was caries incidence (initial and cavitated) in the occlusal fissures of the selected emerging molars and the secondary endpoint was salivary mutans streptococci (MS) counts. After the signed consent and baseline examination, the subjects were stratified with respect to type of molar (age) and degree of eruption and then randomly allocated into a chlorhexidine varnish (CV) group ( $n = 92$ ) and a fluoride varnish (FV) group ( $n = 91$ ) with aid of a computer programme. The CV group received topical applications of a varnish containing 1 % chlorhexidine and 1 % thymol (Cervitec® Plus, Ivoclar Vivadent, Liechtenstein) at baseline and then every third month for 21 months, in total 8 applications. The FV group received topical fluoride varnish applications (0.1 %

**Fig. 1** Flowchart of participants during the trial. *BL* baseline, *CV* chlorhexidine varnish, *FV* fluoride varnish, *FU* follow-up



**Table 1** Baseline eruption stage of the included 1st and 2nd permanent molars in the chlorhexidine/thymol varnish (CV) and the fluoride varnish (FV) groups

Eruption stage after Carvalho et al. (1991)	1st molar		2nd molar	
	CV group	FV group	CV group	FV group
I	27 (47 %)	27 (52 %)	21 (62 %)	23 (59 %)
II	29 (50 %)	23 (44 %)	13 (38 %)	16 (41 %)
III	2 (3 %)	2 (4 %)	0 (0 %)	0 (0 %)

Values in the table denote number of subjects and percent

Stage I = more than half of the occlusal surface covered by gingiva

Stage II = less than half of the occlusal surface covered by gingiva

Stage III = tooth partially erupted without gingival coverage

difluorosilane; Fluor Protector, Ivoclar Vivadent, Liechtenstein) at baseline and after 6, 12 and 18 months. To equalise the number of treatments in both groups, the children of the FV group received placebo varnish applications after 3, 9, 15 and 21 months. The patients and their parents were instructed to immediately report any perceived side effects to the clinical staff.

### Questionnaire

At baseline, the parents completed a questionnaire including information on socio-economic characteristics, demographic features, oral hygiene routines (tooth brushing, fluoride supplements) and dietary habits for each child.

### Clinical procedures

All study subjects were examined by the same experienced dentist at baseline and after 24 months. The dentist was not involved in the treatment of the children and he was not aware of the group allocation. Any decision concerning preventive and restorative treatment was taken by their regular dentist based on individual need. Oral hygiene instructions were given to all participants and their parents at baseline appointment and this information was repeated at the subsequent examinations. All children were encouraged to brush their teeth twice daily with fluoride toothpaste during the course of the study. Cavitated carious lesions were restored prior to the study. The intra-examiner reliability was checked through a re-examination of 30 children from the study group within 1 month after inclusion.

### Caries registration

The examinations were performed after professional tooth cleaning and drying using a dental mirror and a blunt WHO probe. For teeth partly covered with gingiva, the operculum was carefully lifted and the tooth was cleaned by air and water spray for 10 s. Carious lesions were scored on cavity level according to WHO criteria (1997) and the presence of

initial lesions on smooth surfaces and in the fissures (visible white chalky area with no cavitation) was registered. No radiographs were exposed.

### Saliva sampling and analysis

Paraffin-stimulated whole saliva samples were collected at baseline and each follow-up during chewing for 5 min and the salivary MS counts were estimated with CRT<sup>®</sup> chair-side tests according to the manufacturer's manual (Ivoclar Vivadent, Schaan, Liechtenstein). Following incubation (37 °C) in anaerobic conditions for 48 h, the number of colony forming units (cfu) was scored as "low" ( $\leq 10^4$  cfu/ml), "moderate" ( $>10^4 - \leq 10^6$  cfu/ml), or "high" ( $>10^6$  cfu/ml).

### Visible plaque

The presence of dental plaque on the occlusal surface of each erupting permanent molars was recorded according to the criteria by Carvalho et al. (1991): 0 = no visible plaque; 1 = hardly detectable plaque, restricted to grooves and fossae; 2 = plaque easily detectable in grooves and fossae; 3 = the occlusal surface partially or totally covered with heavy plaque accumulations.

### Varnish application

Both varnishes were applied on all teeth by dental assistants after professional cleaning with a slurry of pumice paste and a rotating rubber cup followed by interdental flossing with un-waxed dental floss. After isolating the teeth with cotton rolls and drying with compressed air, a small quantity (0.2–0.4 ml) of the varnish was applied on all accessible tooth surfaces using a micro-brush. Special care was taken to cover the fissures of the selected erupting molars. After 60 s drying of the varnish, the children were advised to refrain from eating and drinking for 1 h and tooth brushing for 24 h. Dental floss was not allowed until the following day.

### Statistical methods

All data were processed with the IBM SPSS Statistics (version 20.0, Chicago, Ill., USA). The Mann–Whitney test was applied to compare non-parametric data between the groups, while the relative risk for caries development in erupting molars was calculated from two-by-two tables. Distributions were compared with Chi-square tests. Cohen's kappa value was calculated for the intra-examiner reliability. A  $p$  value  $< 0.05$  was considered as statistically significant.

The sample size was calculated based on a caries incidence of 25 % and with the risk of type I ( $\alpha$ ) and type II ( $\beta$ ) errors set at 0.05 and 0.2, respectively. To be considered equal in terms of fissure caries development, the difference between the two regimes should not exceed 15 percent, indicating that 80 children would be needed in each arm. To account for attrition, the sample size was increased by 10 %.

## Results

### Study group characteristics

The parents of the children in the study groups were of low/medium socio-economical level; 50 % had a College or University degree and 24 % were immigrants. Fifty percent of the children brushed their teeth with fluoride toothpaste twice a day, while 6 % reported irregular non-daily brushing. The majority (63 %) brushed without parental help. Almost 70 % of the parents had not noticed the eruption of permanent molars. There were no statistically significant differences between the CV and the FV groups at baseline concerning socio-economy, oral hygiene or dietary habits.

### Caries incidence in erupting molars

The mean numbers of decayed and filled surfaces at baseline and after 2 years are shown in Table 2. The mean values were higher in the FV group, both at baseline and after 2 years, but the differences were not statistically significant. During the study period, 11 children developed non-cavitated caries lesions in the 1st permanent molars and two children in the 2nd permanent molars. As shown in Table 3, there was no statistically significant difference between the CV and FV groups (relative risk 1.08; 95 % CI 0.94–1.25). The intra-examiner reliability for the caries registrations (initial and cavitated) was good (Cohen's kappa = 0.80).

### Visible plaque and salivary mutans streptococci counts

At baseline, visible plaque in the pits and fissures was detected in 66 % of the erupting molars, while 17 % had

**Table 2** Mean and standard deviation (SD) scores of dfs/DFS (all teeth) in the chlorhexidine (CV) and fluoride varnish (FV) groups at baseline and after 2 years

Time/caries index	<i>n</i>	CV	FV	<i>p</i>
Group with erupting 1st molars				
Baseline (dfs + DFS)	110	5.8 (8.5)	9.4 (13.0)	NS
2-year FU (dfs + DFS)	98	5.8 (7.6)	8.6 (10.2)	NS
Group with erupting 2nd molars				
Baseline (dfs + DFS)	73	1.8 (3.9)	2.4 (3.7)	NS
2-year FU (DFS)	69	1.7 (4.1)	2.2 (3.8)	NS

NS not statistically significant

**Table 3** Caries incidence in the erupting molars during the 2-year study in the chlorhexidine (CV) and fluoride varnish (FV) groups

Treatment group	New lesions	No lesions	Sum
CV ( <i>n</i> = 92)	5	78	83
FV ( <i>n</i> = 91)	8	76	84
Total	13	154	167

Values in the table denote the number of subjects

RR = 1.08 (95 % CI 0.94–1.25), NS

**Table 4** Distribution of salivary mutans streptococci counts at baseline and the 2-year follow-up (FU) in children with 1st and 2nd molars under eruption in the chlorhexidine (CV) and fluoride varnish (FV) groups

Time	Low levels		Moderate levels		High levels	
	CV	FV	CV	FV	CV	FV
1st molar baseline	26	17	52	48	22	35
1st molar FU	67	36	31	55	2	9*
2nd molar baseline	18	18	65	70	17	12
2nd molar FU	78	30	22	62	0	8*

Values in the table denote the number of subjects

NS not significant

\* Significantly different distribution compared with baseline (Chi-square test) ( $p < 0.05$ )

the occlusal surfaces completely covered with plaque. The distribution of salivary MS counts at baseline and after 2 years is displayed in Table 4. More than 60 % of all children harboured moderate to high levels at baseline. At the 2-year follow-up, significantly lower MS levels were found among the children in the CV group compared with the FV group ( $p < 0.05$ ).

### Adverse effects and acceptability of treatment

No adverse effects were reported during the trial and both varnishes were well tolerated by the trial participants.

## Discussion

The present study was undertaken to compare the effectiveness of an antibacterial and a fluoride varnish to protect permanent molars from fissure caries development during eruption. This comparative design was motivated by the fact that the use of placebo should be limited in modern research for ethical reasons when interventions with proven efficacy are available (Piaggio et al. 2012). The parallel group design was chosen to avoid the risk for uncontrolled cross-over effects that may occur in spit-mouth designs (Twetman 2004). The 3-month application interval of the antibacterial varnish was adopted from a previous trial (Petersson et al. 2000) and the biannual fluoride varnish applications followed the local preventive programme based on the current ADA guidelines (Weyant et al. 2013). The dropout rate over 2 years was fairly low and the compliance with the study protocol was in most cases excellent and well controlled as the intervention mainly was based on professional measures. The effect of the oral hygiene information and dietary advocating provided regularly to all participants and their parents remains unknown but the fact that parents became aware of the presence of erupting molars in their child's mouth, probably played a certain role.

Our main results indicated that the two varnish regimes performed equally concerning the primary endpoint, while only the antibacterial varnish influenced the salivary MS counts. Thus, the null hypothesis could not be rejected however and it must be stressed that the incidence of clinical fissure caries in the erupting molars was lower than expected (<10 %) in both groups over the 2-year study period. This means that the study proved in retrospect to be underpowered which certainly limits the conclusions.

Another shortcoming was that early lesions may already have been present at baseline since practically all teeth were partly covered by gingiva at the time of inclusion. The use of a laser fluorescence device would probably have detected a higher incidence of early fissure caries when compared with the visual–tactile examination used here, although with an increased risk for false-positive diagnosis (Twetman et al. 2013). It should be noted that all new lesions in the permanent molars were non-cavitated and could be managed either by resin fissure sealants or by non-invasive flowable resin composite fillings. Thus, both treatment modes may therefore be considered as equal options to prevent fissures in permanent molars from being decayed during eruption in children with high caries risk. Yet the lower application frequency of fluoride varnish gives this regime a certain clinical advantage. For fully erupted permanent molars, however, fissure sealants with resin-based materials are the first treatment of choice for children with caries risk, provided that perfectly dry

conditions can be obtained (Beauchamp et al. 2008; Schwendicke et al. 2015).

The present observation that topical applications of CHX varnish every third month resulted in significant reductions of salivary MS counts was expected in the light of several previous studies (Araujo et al. 2002; Zhang et al. 2007; Ribeiro et al. 2007). This was probably a reflection of the fact that all accessible surfaces of each of the entire dentitions were covered by the varnish. Site-specific samplings of the emerging molars would have provided more detailed information on the local effects of the varnishes but such samples were not obtained in the present trial. However, a recent study has suggested short-term reductions of MS in dental plaque after intense applications of both a fluoride and a chlorhexidine varnish (Paul et al. 2014), while Sajjan et al. (2013) have published contrasting results. The only previous clinical comparison between the two products used in the present study displayed equal efficacy in the prevention of proximal caries in adolescents (Petersson et al. 2000).

An interesting aspect is that the rationale of using antibacterial agents directed against mutans streptococci recently has been questioned, since caries is not a classical infectious disease but a result from a complex interaction between the commensal microbiota, host susceptibility and environmental factors (Wade 2013). This means that the future prevention of caries probably will move away from treating dental diseases by targeting specific oral pathogens towards an ecological approach aiming to modify the structure and the development of the biofilm (ten Cate and Zaura 2012; Maltz and Beighton 2012). Nevertheless, further well-conducted randomised trials are required before the optimal way to prevent occlusal fissure caries development in permanent molars within the first years of eruption can be established.

## Conclusion

Within the limitations of the present trial we were unable to demonstrate any differences in occlusal caries development in erupting molars following regimes based on quarterly applications of a chlorhexidine/thymol-containing antibacterial varnish or a fluoride varnish. The fluoride varnish required, however, less frequent applications and may, therefore, be more feasible for both professionals and patients.

**Acknowledgments** The varnishes (chlorhexidine/thymol—Cervitec<sup>®</sup> and fluoride—Fluor Protector<sup>®</sup>) and the chair-side tests (CRT bacteria) were generously supplied by Ivoclar Vivadent AG, Schaan, Liechtenstein.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical standards** All procedures performed in the study 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.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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