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Efficacy of HAF toothpastes in primary and permanent dentitions. A 2-years triple-blind RCT

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ABSTRACT

Objectives: The aim of this RCT was to compare the caries preventive efficacy and the slowing down of previous caries lesions of toothpastes containing fluoride biomimetic hydroxyapatite (HA) complex compared to sodium monofluorophosphate fluoridated toothpastes in Italian schoolchildren. To validate this hypothesis a triple-blind randomized clinical trial was designed.
Methods: In total 610 children (4–5 and 6–7 years) were enrolled. Four toothpastes, two containing fluoride-substituted hydroxyapatite (HAF) (1000 and 1450 ppmF) and magnesium-, strontium-, carbonate-substituted hydroxyapatite, in a chitosan matrix and two Mono fluoridated toothpastes (1000 and 1450 ppmF) were randomly administered during 24 months to two groups with younger children (G_{young}) and to two groups with older children (G_{old}), those containing 1450 ppmF. ICDAS was used to score lesions as initial (up to score 2), moderate (scores 3–4) and severe (scores 5–6). The children were instructed to brush for two minutes three times/day. Caries examination was repeated at 12 and 24 months. The efficacy of the treatment was assessed by calculating the reduction in Risk Ratio (RR) and the number needed to treat (NNT). *Results*; Overall, 518 patients (G_{young} = 268; G_{old} = 250) concluded the trial. The drop-rate was 11.84% for G_{young} and 17.22% for G_{old}. The caries increment at 24-month evaluation was statistically lower in the primary dentition in the HAF arms compared to the traditional fluoridated arms (0.18 *vs* 0.27 $p = 0.04$ in G_{young} and 0.16 *vs* 0.30 $p = 0.01$ in G_{old} for severe lesions). In the permanent dentition (G_{old}), caries increase was also statistically lower in the HAF arm, both for initial and severe lesions (0.09 *vs* 0.17 $p = 0.02$ and 0.18 *vs* 0.28 $p = 0.01$, respectively). In primary dentition, children receiving HAF Toothpaste had a RR of 39% (G_{young}) and 38% (G_{old}), compared to children receiving traditional Toothpastes. The RR in the permanent dentition was 29% in children treated with HAF toothpaste. *Conclusions*; The use of toothpastes containing biomimetic hydroxyapatite and fluoride reduces caries increment in children over a period of 2 years more than traditional fluoridated toothpastes.

1. Introduction

Although preventable, dental caries is one of the most prevalent chronic disease among children worldwide, and the most common unmet healthcare need among disadvantage children [1,2]. Preventive strategies are needed and recommended to control caries risk factors,

Abbreviations: HA, Hydroxyapatite; HAF, Fluoride-substitute Hydroxyapatite; ICDAS, International Caries Detection and Assessment System; RR, Risk Ratio; HR, Hazard Ratio; NNT, Number Needed to Treat.

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mainly based enhancing host resistance, *i.e.* twice-daily use of fluoride toothpastes [3,4].

Fluoride (usually *via* toothpaste) has the most consistent benefit in preventing caries development and re-mineralizing initial lesions [5]. Caries still develops in high-risk individuals of all ages, irrespective of the dose of fluoride used [6,7]. Fluoride toothpastes containing less than 1450 ppmF might be less effective in high-risk children [8]; toothpastes containing higher concentrations of fluoride are available in some countries, but required a prescription. Furthermore, high concentrations fluoride products increase the risk of adverse effects such as dental fluorosis [9,10].

One of the new caries remineralizing technologies is the synthetic hydroxyapatite (HA) ($Ca_{10}(PO_4)_3(OH)_2$) as a bioactive-compatible material with similar chemical composition to the apatite crystals of human enamel, inserted as micro-cluster or nanocrystalline forms in different oral care products. Several *in-vitro/situ* studies have provided evidences supporting the caries remineralization and prevention effect of HA when inserted in oral care products, based on its ability to strongly adsorb to tooth surfaces, plaque components and bacteria [11–13]. A toothpaste containing ion-doped hydroxyapatite (Sr-Mg-CO3-HA) and HA partially substituted with fluoride (HAF) embedded in a chitosan matrix showed, *in-vitro,* a promising capacity of restore demineralized enamel [14].

From this premise, a hypothesis was postulated: fluoridated toothpastes containing biomimetic hydroxyapatite complex and HAF are more effective in preventing new carious lesions and slowing down the progression rate of previous lesions compared to traditional fluoridated toothpastes containing sodium monofluorophosphate. The null hypothesis to be tested in this clinical trial was that there was no difference in the effectiveness of the different toothpaste products in preventing new carious lesions and slowing down the progression rate of previous lesions. To validate this hypothesis a triple-blind randomized clinical trial was designed.

2. Materials and methods

The study was designed as a randomized, triple-blind clinical trial, in which the patient, operator, and evaluator were masked to the group assignment. An independent monitor had the code, and it was not broken until the statistical analysis was finalized. A third researcher, not involved in the evaluation process, was responsible for the randomization process.

The study was carried out at the Dental Clinic of the local University, Italy. (Ethical Committee approval written in the statement) and registered at http://www.clinicaltrial.gov (NCT04906291).

2.1. Study population

Italian National Institute for Statistics website (https://www.istat.it) provided the number of children aged 4–7-year-old living in the area as 13,239. The natural fluoride concentration in tap water of the district is 0.04 mg/l (http://www.abbanoa.it/distretto- 6).

Children, whose birthday fell between September 2012 and June 2013 attending kindergarten and children, whose birthday fell between September 2010 and June 2011, attending primary school, were possible participants.

The selection procedures might be summarized as follows:

(1) A leaflet about the study aims was to all the school authorities of the study area ($n = 157$). Sixty-four schools accepted to take part of the trial (positive reply rate 40.76%).
(2) All parents/guardian of children attending the schools that replied positively were contacted by a second leaflet about the trials and asking their children consent to be enrolled to the study.

Each school-class was considered a cluster to facilitate randomization and the carrying out of the trial. Also, the randomization was conducted at school-class level. Randomization was performed using Excel 2014 using systematic cluster sampling; each school-class was identified as a cluster and compiled into a list and four groups (two for each age group) were created. The first cluster was randomly chosen, while the others were selected at the systematic interval of three classes. The number of subjects were approximately the same in each class (range 14–16 children).

For families that decided to not participate into the trial, the children will receive the toothpaste but they were not clinical examined and their data not recorded.

The inclusion criteria were: Written declaration of informed consent signed by parents/guardians; Age between 4 and 5 years and 6–7 years; Good general health (*i.e.* absence of systemic diseases), as assessed by the examiners; Agreement of not to use any oral hygiene products except for the toothpastes provided. The exclusion criteria were: Ongoing oral or dental treatment (*i.e.* restorative or orthodontic treatment) except for emergency treatment, Known allergic reaction to an oral hygiene product and/or medication and/or dental material previously used in the mouth or pharynx; Allergy to one of the components of the test products; Participation in another clinical trial either currently or within the last 30 days; Antibiotic therapy within the past 3 months.

Power analysis was performed using G*Power 3.1.3 for Apple using a non-parametric Mann–Whitney U test, with an effect size of 0.25 and an error probability of 0.05; the number of subjects in each age group was set at 256 (504 subjects in total), with an actual power of 0.95. The sample size was increased by 15% to safeguard the estimates against the possible number of non-responders.

2.2. Study design

The clinical trial was supposed to be carried out from February 2018 to June 2020. Due to the COVID19 pandemic, on 9th March 2020, the Italian Government imposed a national lockdown until 4th May 2020, but schools remained closed until the end of the school-year. During the second year of the trial, the pandemic COVID reached Italy and on 9th March 2020, the government of Italy imposed a national lockdown or quarantine, restricting the movement of the population except for necessity, work, and health circumstances, in response to the growing pandemic of COVID-19 in the country. At this date, 452 (87.26%) children of the enrolled sample had completed the trial. As a consequence, some of the follow-up examinations were performed in September and October 2020. The design of the study (CONSORT flowchart) is displayed in Fig. 1, including a baseline examination prior the trial start, one interim examination after 12 months, and one last at the end of the examination period (24 months in the original study protocol or longer due to the COVID-19 pandemic).

The clinical examination was repeated after 12 months and at the end of the experimental period (24 months or longer due to the COVID-19 pandemic).

Two age groups were created, a younger group (4–5 years at baseline, G_{young}) and an older age group (6–7 years at baseline, G_{old}). At baseline, a standardized questionnaire was administered to parents/caregivers to gain information regarding caries risk factors [15,16] as behavior habits (toothbrush frequency, use of fluoride), dietary habits (use of pacifier at night, number of meals, diet cariogenic content), socio-economic status of the children/family/caregivers, categorized according to the SocFam scale [16] as medium-low, medium and medium-high level, and lifestyle behaviors (frequency of dental check-ups).

2.3. Clinical examination

Subjects were examined at school using a mouth mirror and a WHO—Community Periodontal Index probe under optimal lighting (each examiner wearing a led headlight); the teeth were cleaned and

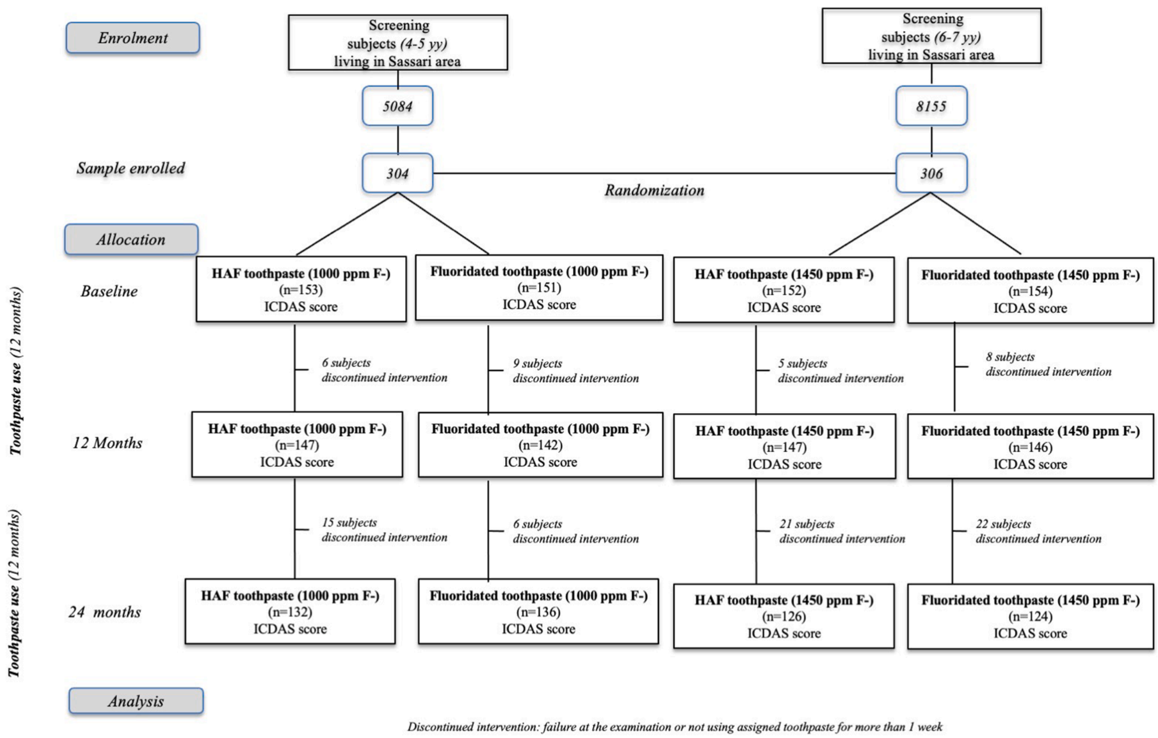

Fig. 1. CONSORT flow-chart of the study design.

dried with a piece of gauze prior of the examination. Caries lesions, the number of filled and missing teeth for caries, were recorded at tooth level using the ICDAS (International Caries Detection and Assessment System) as initial or moderate or extensive lesions [17]. The lesions were defined as follows: lesions having not reached the stage of an established lesion with cavitation, "initial caries" (up to ICDAS scores 2); white or brown spot lesion with localized enamel breakdown or an underlying dentine shadow without visible dentine exposure, "moderate caries" (ICDAS scores 3 and 4); lesions with distinct cavity with visible dentine, "severe caries" (ICDAS scores 5 and 6). All parents/caregivers whose children presented caries lesions received a letter in which a dental visit was recommended due to the presence of carious lesions.

The calibration of examiners (four) was performed prior of the start of the trial and repeated before the interim examination (12 moths) and before the last examination (24 months), the calibration procedure was comparable with the trial conditions. An author acted as benchmark examiner (GC); at baseline the training comprised: 1- a theoretical course (6 h) with a guidance manual; 2- examination of extracted teeth (20 primary/20 permanent) plus a session of 100 photographs of extracted teeth; 3- a clinical training involving the examination of 60 children with a wide range of clinical caries situation. The subjects were re-examined after 72 hrs. Inter-and-intra-examiner reliability was evaluated: the first comparing the benchmark (GC) and the examiners outcomes through fixed-effect analysis of variance; the second as the percentage of agreement using Cohen's kappa statistic [18]. A good inter-examiner agreement was recorded for sound teeth $p = 0.22$, mean square of error = 0.51 $F = 1.45$; for initial lesions $p = 0.25$, mean square of error = 0.47 $F = 1{,}38$; for moderate lesions $p = 0.24$, mean square of error =0.44 $F = 1.36$ for severe lesions and $p = 0.14$ mean square of error = 0.43 $F = 1.84$. Intra-examiners reliability was also quite high (Cohen's kappa = 0.91, 0.89, 0.92 and 0.85 for sound teeth, initial lesions, moderate lesions and severe lesions, respectively). Before interim and the last follow-up examination, calibration was also run and achieved; 30 children not being enrolled into the clinical trial were examined and re-examined after 72 hrs (inter-examiners reliability mean square of error = 0.49 $p = 0.17$ $F = 1.78$ and intra-examiner reliability Cohen's kappa = 0.89). Before the last examination, considering the high values of inter and intra-examiners reliability, only 20 children not enrolled into the trial were examined and re-examined, and quite similar previously found inter- and intra-examiner reliabilities values were confirmed.

2.4. Treatment

Overall, 610 subjects were examined and enrolled in the trial.

All the toothpastes used in the trial were produced and supplied by Curasept S.p.A (Saronno, Italy). The toothpastes were: HAF toothpaste (1000 ppmF) containing fluoride-substituted Hydroxyapatite (HAF) and magnesium-, strontium-, carbonate-substituted hydroxyapatite, in a chitosan matrix; sodium monofluorophosphate fluoridated toothpaste (1000 ppmF) without other active components; HAF toothpaste (1450 ppmF) and sodium monofluorophosphate fluoridated toothpaste (1450 ppmF). The first two toothpastes were administered to the G_{young}, while the latter two to the G_{old}. All tubes were identical in weight and shape and were indicated by a color (red and green) and a number (1 or 2), according to the group.

The children were instructed to brush with a manual toothbrush for at least two minutes after each main meal (three times/day) one at school under the supervision of one teacher and twice at their homes. Two different sizes of toothpastes were indicated: the pea-size in G_{young} and normal full load in G_{old} [19]. The compliance and any observed side effects of the products by means of a questionnaire administered to the participants' parents each month for the duration of the trial. In the same questionnaire was also investigated the possible use of other fluoridated products (*i.e.* salt, gels, mouthwashes) that were not allowed throughout the trial. If a lapse in the use of the allocated toothpaste of more than one week was reported, or if the use of a different fluoridated product was found during a similar period, the child was banned from the trial. To evaluate the success of the trial, participants were given toothpaste necessary for two months at a time and asked to return the empty pack when receiving the new one for the following months. During the lockdown, one of the authors (CS) contacted all the children and the families three times per week via Zoom to check the correct use of the toothpastes.

2.5. Statistical analysis

All the data were input into a spreadsheet (Microsoft Excel 2021 for Mac, version 16.4.8). Statistical analyzes were performed using Stata/SE1 software, version Stata/SE 16.1 for Mac (Intel 64-bit).

The Ekbäck's criteria [20] were modified to analyze the data of the present paper; caries incidence rate was calculated on each tooth (primary and permanent) as the unit of analysis and evaluated using a multi-step approach:

(a) The net caries increment for initial, moderate, and extensive caries severity using ICDAS (Δ-initial, Δ-moderate, and Δ-extensive) was calculated at the 12-months and at more than 24months examinations.
(b) Events were defined as a tooth getting a lesion or as the sum of the Δ-caries changes of status recorded at the baseline examination, at interim, and at the last examination. The number of events was appraised by subtracting the number of caries-free teeth at last examination from those at baseline.

The non- parametric Mann–Whitney U test was applied to assess the differences across mean number of events between groups.

The efficacy of the treatment was assessed for those who fully followed the protocol (per-protocol subjects) by calculating the reduction in risk ratio (RR) and the related number needed to treat (NNT) value [21]. An event was defined as the change of status at tooth level, *i.e.* the development of a new lesion or the progression of an existing lesion to a more severe stage.

Cox Proportional Hazards models were run to assess the factors associated with caries change of status. Estimates are reported in the hazard ratio (HR) and their respective 95% confidence interval (95%CI). The Efron method was used to handle tied failures. The Kaplan–Meier estimator was endorsed to estimate the survival fraction of teeth measured as the change of status during the trial. The Greenwood's formula was used to approximate the variance of the Kaplan-Meier estimator. For all statistical analyzes, the statistical significance was set at $\alpha = 0.05$.

3. Results

Overall, 10,655 teeth ($teeth_{young} = 6024$; $teeth_{old} = 4631$) from 610 children were included in the study. The drop-rate was 11.84% and 17.22% in G_{young} and G_{old}, respectively, with family moving to another area or family/child deciding to leave the trial as the main dropout reasons. The highest rate was recorded in G_{old} between the interim and last examination. So, 518 children (9380 teeth) concluded the experimental period. The main discontinued intervention reasons were the failure of the examination (as shown in Fig. 1).

At baseline, caries measurement data were almost overlapping in the two old and young groups and no gender difference was also observed (*data not shown*). The behavioral, dietary, socio-economic status and dental check-up frequency are reported in Table 1. The caries increment (at interim evaluation) was not statistically significant different in the two age groups, regardless of the type of toothpaste; nevertheless, the caries increment was lower in the HAF arms respect to fluoridated ones in the majority of caries levels, both in primary and permanent dentition (Table 2). The caries increment at the last examination compared to baseline was statistically significantly lower in the primary dentition in the HAF arms compared to the fluoridated arms (0.18 *vs* 0.27 in G_{young} $p = 0.04$ and 0.16 *vs* 0.30 $p = 0.01$ in G_{old} for severe lesions). In the permanent dentition the increase was again statistically significant lower in the HAF arms, both for initial and severe caries lesions (0.09 *vs* 0.17 $p = 0.02$ and 0.18 *vs* 0.28 $p = 0.01$, respectively). In primary dentition, children receiving HAF toothpaste had a reduction of Risk Rate (RR) of 39% in G_{young} and 38% in G_{old}, compared to children receiving Fluoridated toothpastes. The RR in the permanent dentition was 29% in children treated with HAF toothpaste. The Number Needed to Treat (NNT) in primary dentition was 3.97 and 4.43 in G_{young} and G_{old}, respectively; while NNT was 5.68 in permanent dentition (Table 3).

Teeth treated with HAF toothpastes had an overall survival rate of 88.20% (G_{young}), 94.00% (G_{old}) in primary dentition and 94.22% in permanent dentition, while teeth treated with fluoridated toothpastes had a survival rate of 87.90% (G_{young}), 92.82% (G_{old}) for primary dentition and 93.51% for permanent dentition (as shown in Fig. 2). The survival rates were not statistically significant different using both the Wilcoxon and Log-Rank tests ($p = 0.09$ and 0.12 in primary and 0.08 in permanent dentition).

The results of the multivariate analysis (Cox-Regression) are displayed in Table 4. In the HAF toothpastes arms, a significant reduction in the risk rate of caries change of status was highlighted (HR = 0.60 $_{95\%}$CI = 0.45/0.84 and HR = 0.88 $_{95\%}$CI = 0.61/0.97 in younger and older groups, respectively). In fluoridated toothpastes arms, the caries change of status was not associated to the treatment in G_{young}, while in G_{old} a statistically significant effect of reduction of risk rate of caries change of status was observed (HR = 0.97 $_{95\%}$CI = 0.82/1.01). The presence of moderate caries lesions (HR = 0.71 $_{95\%}$CI = 0.57/0.91in G_{young} and HR = 1.15 $_{95\%}$CI = 1.01/1.54 in G_{old}, respectively), the use of pacifier at night, (HR = 1.81 $_{95\%}$CI = 1.23/2.52 in G_{young} and HR = 1.60 $_{95\%}$CI = 1.22/3.00 in G_{old}) cariogenic diet (HR = 2.01 $_{95\%}$CI = 1.18/3.01 in G_{young} and HR = 1.74 $_{95\%}$CI = 1.20/3.15 in G_{old}) were statistically significant associated to caries change of status. In older groups, going for dental check-ups only in case of problem was also statistically associated to the caries change of status (HR = 1.28 $_{95\%}$CI = 1.09/2.05). Otherwise, on both age groups a Medium-High SES has a preventive effect with a reduction of the risk of caries change of status, whereas the use of fluoridated toothpaste had a preventive effect in the younger group only.

Table 1
Oral Hygiene habits, dietary behaviors, socio-economic status and dental check-up frequency of the children enrolled in the trial in the two groups.

	4–5 years *n (%)*		6–7 years *n (%)*	
	HAF (1000ppmF-)	*F (1000ppmF-)*	*HAF (1450ppmF-)*	*F (1450ppmF-)*
Toothbrush frequency				
<2 a day	68 (22.37)	72 (23.68)	26 (8.50)	28 (9.15)
>2 a day	85 (27.96)	79 (25.99)	126 (41.12)	126 (41.12)
Use of fluoridated toothpaste				
No	44 (14.47)	42 (13.81)	13 (4.25)	14 (4.58)
Yes	109 (35.86)	109 (35.86)	139 (45.42)	140 (45.75)
Use of pacifier at night				
No	64 (21.05)	61 (20.07)	145 (47.39)	143 (46.73)
Yes	89 (29.28)	90 (29.60)	7 (2.29)	11 (3.59)
Main meals frequency				
2 a day	7 (2.30)	8 (2.63)	15 (4.90)	12 (3.92)
3 a day	42 (13.82)	39 (12.83)	130 (42.48)	135 (44.12)
>3 a day	104 (34.21)	104 (34.21)	7 (2.29)	7 (2.29)
Cariogenic diet				
No	7 (2.30)	7 (2.30)	19 (6.21)	23 (7.52)
yes	146 (48.03)	144 (47.37)	133 (42.48)	131 (44.12)
Socio-economic status				
Medium-Low	87 (28.62)	81 (26.64)	66 (21.57)	67 (21.90)
Medium	48 (15.79)	44 (14.47)	63 (20.59)	57 (18.63)
Medium-High	18 (5.93)	26 (8.55)	23 (7.52)	30 (9.80)
Dental check-ups frequency (parents/caregivers)				
In case of problem	96 (31.58)	95 (31.25)	93 (30.39)	87 (28.43)
Rarely	40 (13.16)	36 (11.84)	41 (13.40)	43 (14.05)
Routinely	17 (5.60)	20 (6.58)	22 (7.19)	20 (6.54)

Table 2
Caries data (ICDAS) as the mean number of caries teeth *per* subject for each ICDAS merged categories at baseline and increment (Δ) between baseline and 12-month examination and between baseline and 24-month examination. Differences between groups were evaluated using the nonparametric Mann–Whitney U test.

Primary dentition younger children (G_{young})									
	Caries teeth at baseline			**Δ0–12 months caries teeth increment**			**Δ0–>24 months caries teeth increment**		
Toothpastes	*mean ± SD*			*mean ± SD*			*mean ± SD*		
	Initial	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*
HAF (1000 ppm F-)	*0.52 ± 0.74*	*0.24 ± 0.36*	*0.34 ± 0.56*	*0.04 ± 0.10*	*0.07 ± 0.14*	*0.11 ± 0.21*	*0.08 ± 0.18*	*0.18 ± 0.22*	*0.18 ± 0.48*
F (1000 ppm F-)	*0.53 ± 0.84*	*0.25 ± 0.34*	*0.35 ± 0.60*	*0.06 ± 0.11*	*0.08 ± 0.16*	*0.14 ± 0.20*	*0.10 ± 0.21*	*0.22 ± 0.26*	*0.27 ± 0.50*
Mann–Whitney U test									
p-value	*0.88*	*0.73*	*0.70*	*0.64*	*0.71*	*0.11*	*0.12*	*0.09*	***0.04***
Primary dentition younger children (G_{old})									
	Caries teeth at baseline			**Δ0–12 months caries teeth increment**			**Δ0–>24 months caries teeth increment**		
Toothpastes	*mean ± SD*			*mean ± SD*			*mean ± SD*		
	Initial	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*
HAF (1450 ppm F-)	*0.62 ± 0.73*	*0.32 ± 0.34*	*0.38 ± 0.46*	*0.06 ± 0.11*	*0.10 ± 0.13*	*0.09 ± 0.20*	*0.08 ± 0.14*	*0.14 ± 0.21*	*0.16 ± 0.34*
F (1450 ppm F-)	*0.58 ± 0.70*	*0.31 ± 0.44*	*0.37 ± 0.54*	*0.06 ± 0.10*	*0.10 ± 0.17*	*0.15 ± 0.28*	*0.14 ± 0.22*	*0.17 ± 0.26*	*0.30 ± 0.51*
Mann–Whitney U test									
p-value	*0.64*	*0.81*	*0.75*	*0.81*	*0.96*	*0.07*	*0.07*	*0.29*	***0.01***
Permanent dentition younger children (G_{old})									
	Caries teeth at baseline			**Δ0–12 months caries teeth increment**			**Δ0–>24 months caries teeth increment**		
Toothpastes	*mean ± SD*			*mean ± SD*			*mean ± SD*		
	Initial	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*	*Initial*	*Moderate*	*Severe*
HAF (1450 ppm F-)	*0.31 ± 0.41*	*0.24 ± 0.41*	*0.19 ± 0.74*	*0.05 ± 0.15*	*0.06 ± 0.24*	*0.07 ± 0.16*	*0.09 ± 0.45*	*0.13 ± 0.28*	*0.18 ± 0.34*
***F* ± 1450 ppm F-)**	*0.39 ± 0.33*	*0.21 ± 0.40*	*0.17 ± 0.42*	*0.06 ± 0.17*	*0.06 ± 0.32*	*0.10 ± 0.18*	*0.17 ± 0.49*	*0.13 ± 0.25*	*0.28 ± 0.58*
Mann–Whitney U test									
p-value	*0.65*	*0.11*	*0.68*	*0.81*	*0.94*	*0.31*	***0.02***	*0.90*	***0.01***

Table 3
Effectiveness of the treatments. An event was defined as the change of status at tooth level.

	Events	Non Events	Event Rate
Younger group (Primary dentition)			
HAF toothpaste (1000 ppm F-)	*790*	*1607*	*0.33*
Fluoridated toothpaste (1000 ppm F-)	*1482*	*1066*	*0.58*
z = 8.45 ***p < 0.01*** *RR = 0.57* $_{95\%}$*CI = 0.46/0.82*	*NNT = 3.97*	$_{95\%}$*CI = 1.95/7.05*	
Older group (Primary dentition)			
HAF toothpaste (1450 ppm F-)	*681*	*938*	*0.42*
Fluoridated toothpaste (1450 ppm F-)	*994*	*544*	*0.64*
z = 8.64 ***p < 0.01*** *RR = 0.66* $_{95\%}$*CI = 0.45/0.80*	*NNT = 4.43*	$_{95\%}$*CI = 1.34/6.91*	
Older group (Permanent dentition)			
HAF toothpaste (1450 ppm F-)	*212*	*388*	*0.35*
Fluoridated toothpaste (1450 ppm F-)	*399*	*279*	*0.53*
z = 7.41 ***p < 0.01*** *RR = 0.67* $_{95\%}$*CI = 0.49/0.72*	*NNT = 5.68*	$_{95\%}$*CI = 2.59/11.41*	

4. Discussion

This triple-blind RCT was aimed to validate the hypothesis that toothpastes containing fluoride biomimetic hydroxyapatite complex (HAF) might be more effective in preventing the development of new carious lesions and/or arrest the progression rate of pre-existing lesions compared to fluoridated toothpastes containing sodium monofluorophosphate. In a comprehensive way, this trial proved that traditional fluoridated toothpastes (1450 ppmF) and HAF toothpastes (1000/1450 ppmF) showed a statistically significant efficacy in caries prevention and progression. Only the product containing the lower amount of sodium monofluorophosphate did not reach a statistically significant efficacy compared to HAF toothpaste containing an equal fluoride content. For clarity, the trial found that the toothpastes containing biomimetic HAF, fluoride and chitosan seems to be clinically effective in both age groups, while fluoridated toothpastes containing sodium monofluorophosphate had a slight statistically significant effect only in the older group in which a more amount of fluoride was used.

Despite caries being a preventable disease and fluoride toothpaste acting as the main means to prevent it, in children disease prevalence is still a major public health problem [16,22]. Caries preventive programs using different strategies were carried out in many countries to decrease caries prevalence and incidence [3,23,24]. Nevertheless, the costs of caries prevention are not achievable for the majority of countries worldwide, as it happens in Italy, where the only method for caries prevention available for the majority of children is through oral hygiene maintenance [15,16,25].

Due to the nature of the disease, it is necessary to prevent controlling the contributing factors. Fluoridated toothpastes increase hard tissues remineralization and reduces plaque-pH fall, decreasing microorganisms in the dental plaque [6]. Traditionally, three fluoride salts are added in toothpastes, stannous fluoride (SnF_2), sodium monofluorophosphate (Na_2PO_3F), and sodium fluoride (NaF). Although all salts are effective, it is difficult to establish which is the most effective since different conclusions are available in literature starting from the same premises [26]. The efficacy of sodium monofluorophosphate at 1450 ppm F was confirmed both in primary and permanent dentitions by the findings of the present trial, considering the low rate of changes of tooth status during a 24-month period in subjects. Nevertheless, a lower amount of fluoride (1000 ppmF) was not sufficient to control caries development and progression as a single preventive strategy.

Biomimetic HA doped with $CO32^-$, $F-$, Mg^{2+}, and Sr^{2+} ions has shown the *in vitro* capacity to fill enamel interprismatic spaces, restoring the mineral loss [14]. A nanostructured hydroxyapatite toothpaste not containing fluoride has shown the ability to produce, after 15-day treatment, a biomimetic coating on the enamel surface of primary teeth consisting of a new layer of apatite with a high potential of enamel remineralization [27]. These and other findings reported in the literature on the "restorative action" of biometric hydroxyapatites might explain the greater efficacy in caries prevention and in controlling tooth status modification demonstrated in this trial by HAF toothpastes compared to traditional toothpastes containing equal fluoride content.

Chitosan is a biopolymer obtained from the hard skeleton of shellfish or fungi, showing a broad-spectrum of antimicrobial activity. Chitosan-containing toothpaste have been reported better properties in terms of antimicrobial activity (broader antimicrobial spectrum of action and prolonger effect) if compared to traditional toothpastes containing other antibacterial compound or fluoride [28]. This antibacterial effect of chitosan on cariogenic microorganisms, especially mutans streptococci, may have contributed to the described results.

A limitation of this RCT study can be due to the postponement of last

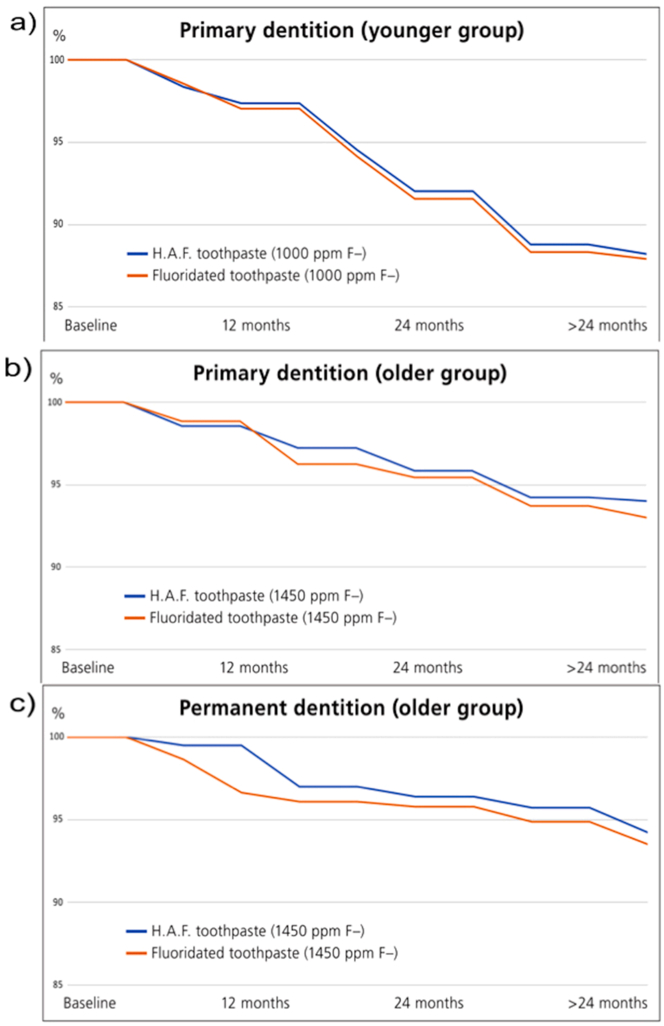

Fig. 2. Survival curves as the change of status at tooth level during the trial: (a) primary dentition in younger groups, (b) primary dentition in older groups and (c) permanent dentition in older groups.

follow-up evaluation due to the COVID-19 pandemic. The absence from a regular school activity may have resulted in less adherence to the protocol and above all in the lack of a supervised toothbrushing, which could affect the results. Furthermore, the brushing, and therefore the use of the different toothpastes, was performed two times/day at home, without the possibility of a control by the authors of a regular adherence to the protocol and one time only at school, under the teachers' supervision. However, this trial present significant strengths: the long period of follow-up, the wide sample size and the evaluation of both primary and permanent teeth represent strengths that make the reliability of the study high. The study design is also a plus of the paper. The choose of a cluster randomization procedure, considering each class as cluster and having distributed the toothpaste to all the children in each class, even those that had not agreed to participate, helped to motivate children participation for a so long period of time.

Fluoride toothpastes are still the most effective and affordable means of preventing caries, especially in countries where other preventive strategies at community level of certain efficacy, such as water fluoridation, are not implemented. Toothpastes with new compounds with remineralizing and antibacterial properties might contribute together with fluoride to reduce a disease, dental caries, which still remains in many countries a public health problem without an adequate response by the competent Health Authorities.

Clinical significance

The outcomes of this trial allow pediatric dentists and Health authorities to select toothpastes containing new compounds with remineralizing, antibacterial properties and fluoride, as active therapeutic agent to reduce dental caries.

Data availability statement

The dataset generated during and/or analyzed during the current study are not publicly available due to data holder restrictions. The data are available on request.

CRediT authorship contribution statement

Maria Grazia Cagetti: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Fabio Cocco:** Conceptualization, Visualization, Writing – original draft, Writing – review &

Table 4
Cox proportional hazards regressions, estimating at tooth level the caries change of status in the two age groups.

	Younger group			*Older group*		
	HR (SE)	$_{95\%}$Confidence Interval	p-value	HR (SE)	$_{95\%}$Confidence Interval	p-value
HAF toothpaste (1000/1450 ppmF)	**0.60 (0.14)**	**0.45–0.84**	**<0.01**	**0.88 (0.10)**	**0.61–0.97**	**0.04**
Fluoridated toothpaste (1000/1450 ppmF)	0.94 (0.22)	0.75–1.04	0.06	**0.97 (0.20)**	**0.82–1.01**	**0.05**
Baseline Variables						
Initial Caries lesions	1.03 (0.12)	0.92–1.11	0.05	1.05 (0.14)	0.93–1.08	0.05
Moderate Caries lesions	1.20 (0.18)	0.97–1.38	0.06	**1.15 (0.16)**	**1.01–1.54**	**0.04**
Severe Caries lesions	**1.21 (0.24)**	**1.04–1.38**	**0.02**	1.11 (0.28)	0.99–1.30	0.12
Toothbrush frequency >2 a day	0.79 (0.26)	0.54–1.15	0.06	0.94 (0.21)	0.62–1.33	0.15
Use of fluoridated toothpaste	**0.67 (0.36)**	**0.44–0.84**	**<0.01**	0.83 (0.30)	0.68–1.45	0.14
Use of pacifier at night	**1.81 (0.33)**	**1.23–2.52**	**0.03**	**1.60 (0.30)**	**1.22–3.00**	**0.01**
2 main meals a day	0.85 (0.12)	0.71 –1.08	0.05	0.91 (0.16)	0.74 –1.10	0.05
3 main meals a day	0.94 (0.20)	0.90–1.22	0.07	0.97 (0.18)	0.85–1.31	0.08
>3 main meals a day	1.14 (0.15)	0.93–1.40	0.06	1.03 (0.16)	0.77–1.66	0.17
Cariogenic diet	**2.01 (0.27)**	**1.18–3.01**	**<0.01**	**1.46 (0.21)**	**1.01–1.69**	**0.04**
Medium-Low SES	**1.65 (0.31)**	**1.08–2.29**	**0.03**	**1.74 (0.28)**	**1.20–3.15**	**0.02**
Medium SES	0.98 (0.21)	0.75–1.34	0.16	1.05 (0.16)	0.71–1.11	0.09
Medium-High SES	**0.71 (0.18)**	**0.57–0.91**	**0.02**	**0.80 (0.26)**	**0.61–0.90**	**0.02**
Dental check-ups (in case of problem)	1.65 (0.27)	0.89–2.70	0.11	**1.28 (0.23)**	**1.09–2.05**	**0.01**
Dental check-ups (rarely)	1.48 (0.24)	0.84–2.06	0.14	1.28 (0.22)	0.88–2.02	0.13
Dental check-ups (routinely)	0.86 (0.15)	0.75–1.05	0.08	0.84 (0.14)	0.76–1.02	0.05
Number of teeth	5350			5905		
Number of children	268			250		

editing, Funding acquisition, Formal analysis. **Richard Johannes Wierichs:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Thomas Gerhard Wolf:** Conceptualization, Writing – original draft, Writing – review & editing. **Claudia Salerno:** Conceptualization, Writing – original draft, Writing – review & editing. **Antonella Arghittu:** Conceptualization, Data curation, Writing – review & editing. **Guglielmo Campus:** Conceptualization, Visualization, Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None.

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References

[1] R.H. Selwitz, A.I. Ismail, N. Pitts, Dental caries, Lancet 369 (9555) (2007) 51–59, https://doi.org/10.1016/S0140-6736(07)60031-2.
[2] V. Machiulskiene, Campus G, J.C. Carvalho, I. Dige, K.R. Ekstrand, A. Jablonski-Momeni, M. Maltz, D.J. Manton, Martignon S, E.A. Martinez-Mier, N.B. Pitts, A. G. Schulte, C.H. Splieth, L.M.A. Tenuta, A. Ferreira Zandona, B. Nyvad, Terminology of dental caries and dental caries management: consensus report of a workshop organized by ORCA and cariology research group of IADR, Caries Res. 54 (1) (2020) 7–14, https://doi.org/10.1159/000503309.
[3] S. Kumar, N.W.Johnson Tadakamadla, Effect of toothbrushing frequency on incidence and increment of dental caries: a systematic review and meta-analysis, J. Dent. Res. 95 (11) (2016) 1230–1236, https://doi.org/10.1177/0022034516655315.
[4] F. Schwendicke, C.E. Dörfer, P. Schlattmann, L.Foster Page, W.M. Thomson, S. Paris, Socioeconomic inequality and caries: a systematic review and meta-analysis, J. Dent. Res. 94 (1) (2019) 10–18, https://doi.org/10.1177/0022034514557546.
[5] J. Winter, M. Glaser, M. Heinzel-Gutenbrunner, K. Pieper, Association of caries increment in preschool children with nutritional and preventive variables, Clin. Oral Investig. 19 (8) (2015) 1913–1919, https://doi.org/10.1007/s00784-015-1419-2.
[6] V.C.C. Marinho, J. Higgins, S. Logan, A. Sheiham, Fluoride toothpastes for preventing dental caries in children and adolescents, Cochrane Database Syst. Rev. 1 (2003), CD002278, https://doi.org/10.1002/14651858.CD002278.
[7] B.T. Amaechi, P.A. AbdulAzees, D.O. Alshareif, M.A. Shehata, P.P.C.S. Lima, A. Abdollahi, P.S. Kalkhorani, V. Evans, Comparative efficacy of a hydroxyapatite and a fluoride toothpaste for prevention and remineralization of dental caries in children, BDJ Open 5 (2019) 18, https://doi.org/10.1038/s41405-019-0026-8.
[8] D.C. Souza, M. Maltz, L.N. Hashizume, Fluoride retention in saliva and in dental biofilm after different home-use fluoride treatments, Braz. Oral Res. 28 (2014) S1806–83242014000100248, https://doi.org/10.1590/1807-3107bor-2014.vol28.0039.
[9] B.T. Amaechi, C. van Loveren, Fluorides and non-fluoride remineralization systems, Monogr. Oral Sci. 23 (2013) 15–26, https://doi.org/10.1159/000350458.
[10] K.J. Toumba, S. Twetman, C. Splieth, C. Parnell, C. van Loveren, N.A. Lygidakis, Guidelines on the use of fluoride for caries prevention in children: an updated EAPD policy document, Eur. Arch. Paediatr. Dent. 20 (6) (2019) 507–516, https://doi.org/10.1007/s40368-019-00464-2.
[11] M. Esteves-Oliveira, N.M. Santos, H. Meyer-Lueckel, R.J. Wierichs, J.A. Rodrigues, Caries-preventive effect of anti-erosive and nano-hydroxyapatite-containing toothpastes *in vitro*, Clin. Oral Investig. 21 (1) (2017) 291–300, https://doi.org/10.1007/s00784-016-1789-0.
[12] H. Meyer-Lueckel, R.J. Wierichs, T. Schellwien, S. Paris, Remineralizing efficacy of a CPP-ACP cream on enamel caries lesions *in situ*, Caries Res. 49 (1) (2015) 56–62, https://doi.org/10.1159/000363073.
[13] B.M. Souza, L.P. Comar, M. Vertuan, C.Fernandes Neto, M.A. Buzalaf, A. C. Magalhaes, Effect of an experimental paste with hydroxyapatite nanoparticles and fluoride on dental demineralisation and remineralisation *in situ*, Caries Res. 49 (5) (2005) 499–507, https://doi.org/10.1159/000438466.
[14] L. Degli Esposti, A.C. Ionescu, E. Brambilla, A. Tampieri, M. Iafisco, Characterization of a toothpaste containing bioactive hydroxyapatites and *in vitro* evaluation of its efficacy to remineralize enamel and to occlude dentinal tubules, Materials 13 (13) (2020) 2928, https://doi.org/10.3390/ma13132928 (Basel).
[15] G. Campus, G. Solinas, A. Sanna, C. Maida, P. Castiglia, Determinants of ECC in Sardinian preschool children, Community Dent. Health 24 (4) (2007) 253–256.
[16] G. Campus, F. Cocco, L. Strohmenger, M.G. Cagetti, Caries severity and socioeconomic inequalities in a nationwide setting: data from the Italian National pathfinder in 12-years children, Sci. Rep. 10 (1) (2020) 15622, https://doi.org/10.1038/s41598-020-72403-x.
[17] N.B. Pitts, K.R. Ekstrand, ICDAS. Foundation, International caries detection and assessment system (ICDAS) and its international caries classification and management system (ICCMS)—methods for staging of the caries process and enabling dentists to manage caries, Community Dent. Oral Epidemiol. 41 (1) (2013) e41–e52, https://doi.org/10.1111/cdoe.12025.
[18] G. Campus, F. Cocco, L. Ottolenghi, M.G. Cagetti, Comparison of ICDAS, CAST, Nyvad's criteria, and WHO-DMFT for caries detection in a sample of italian schoolchildren, Int. J. Environ. Res. Public Health 16 (21) (2019) 4120, https://doi.org/10.3390/ijerph16214120.
[19] G. Thornton-Evans, M.L. Junger, M. Lin, L. Wei, L. Espinoza, E. Beltran-Aguilar, Use of toothpaste and toothbrushing patterns among children and adolescents - United States, 2013–2016, MMWR Morb. Mortal. Wkly. Rep. 68 (4) (2019) 87–90, https://doi.org/10.15585/mmwr.mm6804a3.
[20] G. Ekbäck, S. Ordell, M. Palmetun-Ekbäck, G. Ekbäck, L. Unell, A.K. Johansson, Reporting dental caries disease in longitudinal studies—a suggestion, Swed. Dent. J. 40 (2) (2016) 173–179.
[21] A. Laupacis, D.L. Sackett, R.S. Roberts, An assessment of clinically useful measures of the consequences of treatment, N. Engl. J. Med. 318 (26) (1988) 1728–1733, https://doi.org/10.1056/NEJM198806303182605.
[22] J. Diaz-Nicolas, M.G. Silva-Vetri, S. Rivas-Tumanyan, M.J. Toro, A.R. Elías-Boneta, Prevalence of dental caries in 12-year-olds in San Pedro de Macorís, P R. Health Sci. J. 39 (2) (2020) 210–215.
[23] C. Stein, N.M.L. Santos, J.B. Hilgert, F.N. Hugo, Effectiveness of oral health education on oral hygiene and dental caries in schoolchildren: systematic review and meta-analysis, Community Dent. Oral Epidemiol. 46 (1) (2018) 30–37, https://doi.org/10.1111/cdoe.12325.
[24] N. Fraihat, S. Madae'en, Z. Bencze, A. Herczeg, O. Varga, Clinical effectiveness and cost-effectiveness of oral-health promotion in dental caries prevention among children: systematic review and meta-analysis, Int. J. Environ. Res. Public Health 16 (15) (2019) 2668, https://doi.org/10.3390/ijerph16152668.
[25] M.G. Cagetti, G. Congiu, F. Cocco, G. Meloni, S. Sale, G. Campus, Are distinctive risk indicators associated with different stages of caries in children? A cross-sectional study, BMC Public Health 16 (1) (2016) 12, https://doi.org/10.1186/s12889-016-3865-4.
[26] D.T. Zero, Dentifrices, mouthwashes, and remineralization/caries arrestment strategies, BMC Oral Health 1 (Suppl 1) (2006) S9, https://doi.org/10.1186/1472-6831-6-S1-S9.
[27] M. Bossù, M. Saccucci, A. Salucci, G. Di Giorgio, E. Bruni, D. Uccelletti, M.S. Sarto, G. Familiari, M. Relucenti, A. Polimeni, Enamel remineralization and repair results of Biomimetic Hydroxyapatite toothpaste on deciduous teeth: an effective option to fluoride toothpaste, J. Nanobiotechnol. 17 (1) (2019) 17, https://doi.org/10.1159/000350458.
[28] A.H.M. Resende, J.M. Farias, D.D.B. Silva, R.D. Rufino, J.M. Luna, T.C. M. Stamford, L.A. Sarubbo, Application of biosurfactants and chitosan in toothpaste formulation, Colloids Surf. B Biointerfaces 181 (2019) 77–84, https://doi.org/10.1016/j.colsurfb.2019.05.032.