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Effect of casein phosphopeptide amorphous calcium fluoride phosphate and calcium glycerophosphate on incisors with molar-incisor hypomineralization: A cross-over, randomized clinical trial

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Abstract.
BACKGROUND: Within the scope of minimally invasive dentistry, the use of different biocompatible remineralization agents on incisors affected by molar-incisor hypomineralization (MIH) gains importance.
OBJECTIVE: To evaluate the effect of casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) and calcium glycerophosphate (CaGP) in mineral density (MD) of white/creamy and yellow/brown demarcated opacities on incisors affected by MIH by means laser fluorescence (LF).
METHODS: As a cross-over, randomized trial, twenty-two children with 167 incisors affected by MIH were recruited and randomly assigned to one of the two different agents and crossed over to other agents with two weeks washout in between. Incisors were examined by using LF at all before and after three months periods.
RESULTS: The results of the paired *t*-tests for determining the period effect between the baseline findings showed significant difference in white/creamy and yellow/brown demarcated opacities of LF values for both groups ($p < 0.05$). The difference between both groups according to after categorization of 20% increasing in MD in the percent of change before and after application on LF values; was not found statistically significant in white/creamy ($p = 0.970$) and yellow/brown ($p = 0.948$) opacities.

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CONCLUSIONS: The primary outcome was CPP-ACFP and CaGP had a positive effect in decreasing hypomineralization on MIH-affected enamel for three months period.

Keywords: Molar incisor hypomineralization, minimally invasive dentistry, remineralization, preventive dentistry, casein phosphopeptide amorphous calcium fluoride phosphate, calcium glycerophosphate

1. Introduction

Molar–incisor hypomineralization (MIH) is a type of a lesion as qualitative and developmental enamel defects in the maturation stage of amelogenesis of systemic origin affecting one or more permanent first molars, frequently in combination with demarcated opacities in permanent incisors [1,2]. MIH, a common clinical condition affecting 1-in-5 children worldwide [3], is a principal risk factor for childhood tooth decay for which the etiology is still not completely understood, although several theories have been suggested [4].

MIH-affected enamel appears as well-demarcated opacities inside of healthy enamel. These hypomineralized areas which are frequently visible to the naked eye and diversify in color changes from white/creamy to yellow/brown [5]. Two distinct changes in microstructure were observed in studies evaluating MIH-affected enamel by transmission electron microscopy: less dense enamel prism texture and wider sheath regions [6]. Furthermore, there is a significant reduction in calcium levels in hypomineralized enamel [7]. Even the concentration of phosphorus decreases in porous zones but not to the same level as calcium, leading to a remarkable reducing of the calcium/phosphorus ratio in these areas [6,7].

Different treatment approaches used were desensitizing and remineralizing products, resin infiltration, fissure sealant, atraumatic restorative treatment, resin composite restoration, and stainless-steel crown, but also extraction associated with orthodontic treatment of the permanent first molars was reported on [4]. In this context, new agents with remineralization potential phosphate-origin associated or not to fluoride have been studied, thus promoting to the increasing mineral density (MD) in enamel [8,9]. Many of the published research and guidelines involve the application of remineralizing agents such as high concentration fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) and some new technologies for remineralization of hypomineralized lesions [10–12].

CPP-ACP complex breaks when there is a decline in the pH of the oral cavity. ACP has the ability to release calcium and phosphate ions (and fluoride ions in CPP-ACFP) in the biofilm to preserve a supersaturated circumstance, thuswise increasing the MD in situations with demineralization and/or hypomineralization [13,14].

Another phosphate-based agent with remineralization effect, calcium glycerophosphate (CaGP), is an organic phosphate which has affinity to enamel [15,16]. CaGP, which acts by increasing minerals, is used in the prevention and remineralization of non-carious lesions. It is thought that its mechanism is based on the release of calcium and phosphate ions and thus can be used in the treatment of demineralized/hypomineralized lesions [17].

The objective of this cross-over, double-blind, randomized, clinical study was to evaluate the effect of CPP-ACFP and CaGP on white/creamy and yellow/brown demarcated opacities in MIH-affected permanent incisors by means laser fluorescence (LF) after three months usage.

2. Materials and methods

2.1. Study setting

This cross-over, randomized controlled trial (RCT) with single-arm (1:1 ratio) was approved by the Marmara University School of Dentistry Clinical Research Ethical Committee. It was performed with children whose informed written consent form were signed by their parents. The protocol followed SPIRIT Statement. The study was conducted with all ethical principles of Declaration of Helsinki of 1975, as revised in 2008.

2.2. Samples collection and processing

The study comprised 22 patients, aged 8–12 years, from a pool consulting to the School of Dentistry Pediatric Dentistry Clinic, from August 2019 to February 2020. At a routine appointment, eligible patients who met the inclusion criteria were invited to participate in the study by the lead clinician (BS).

Inclusion criteria were: aged from 8 to 12 years old; clinical diagnosis of MIH; good general health and adequate oral health; residing in a fluoridated area (between 0.10–0.20 mg/L); presence of permanent incisors fully erupted with white/creamy or yellow/brown demarcated opacity, without caries and/or enamel breakdown, and having no previous preventive or restorative treatment; understanding and acceptance of the trial; written agreement of parents for their child's participation in the trial.

Exclusion criteria were: allergic reaction to milk proteins and benzoate preservatives before; uncooperative patient; inability to comply with protocol; patients with systemic disease and/or syndrome, unable to maintain adequate oral hygiene; taking or had taken antibiotics in the last three months; previous fluoride application in the last three months or previous restorations or caries on affected permanent incisors.

2.3. Interventions

This study was carried out for 28 weeks, including a two-week lead-in period, 12-week first phase (A/B), two-week washout period (mid-trial) and 12-week second phase (B/A) during which eligible participants were used to one of the following two remineralization agents in a randomized cross-over order with a lead-in and mid-trial wash-out phases will be allocated to one of the two study groups.

2.3.1. Groups

Group control: (A) Standard fluoride-containing toothpaste (Colgate Total containing 1450 ppm fluoride) in combination with topically applied MI Paste Plus™ [CPP-ACFP; 10% CPP-ACP plus 0.2% NaF (sodium fluoride), GC Corporation Europe].

Group test: (B) Standard fluoride-containing toothpaste (Colgate Total containing 1450 ppm fluoride) in combination with topically applied R.O.C.S.® Medical Minerals Gel (CaGP, magnesium and xylitol, R.O.C.S. Trading GmbH, Munich, Germany).

2.3.2. Discontinuation of intervention

All participants were advised to apply directly to the teeth after brushing for a total of five minutes (as per the manufacturer's instructions). Seven days prior to and during the experimental period, including washout periods, the volunteers brushed their teeth with standardized fluoride-containing toothpaste

(Colgate Total containing 1450 ppm fluoride -as NaF-). The volunteers were asked not to use other fluoride products.

Participants were also provided with the standard fluoride toothpaste and toothbrush and instructed to brush their teeth for two minutes twice a day. The participants were asked not to use any other toothpaste or mouth rinses during this trial. Written instructions for each intervention, which included standardized oral hygiene procedures were given to all children and parents. The dietary advice provided was consistent with that provided for all patients. Intervention A and B products were weighed by the principal investigator (BS) before being provided to the participant. The participants were requested to bring the tubes of product with them on their following review appointment. The tubes were then re-weighed to assess the amount used in the 12-week period, and therefore give a suggestion of compliance.

2.3.3. Compliance on intervention

A diairy has been completed daily by participants to verify adherence to the protocol.

2.3.4. Concomitant care

Study participants will continue to receive the routine medical/pulmonological care they require, according to individual requirements, under the care of their physician(s).

2.4. Outcome

Classification: MIH is diagnosed if at least one first permanent molar is affected by demarcated opacity, post-eruptive enamel breakdown, or atypical caries or restoration on the occlusal and buccal surfaces [18].

Primary outcome measure: Change from baseline on white/creamy and yellow/brown demarcated opacities in MIH-affected permanent incisors, on the 12-week of each stage, evaluating measurements were taken at the endpoint of both first phase (first 12-week application) and second phase (second 12-week application). LF was performed to assess changes in MD.

Participant timeline is described in Fig. 1 (Flow diagram of study-participants' timeline).

2.5. Sample size

The sample size was estimated based on data from a previous study conducted by Biondi et al. [11]. In that study, a mean difference between baseline and 45 days lesion was 2 with a standard deviation of 5. For paired t test, we required total of 51 teeth to achieve a power of 0.80 at a 0.05 significance level. The sample size calculation was performed using MedCalc Version 19.6.4 (MedCalc Software, Ostend, Belgium).

2.6. Recruitement

Participants are recruited through the Marmara University School of Dentistry Pediatric Dentistry Clinic with a validated MIH diagnosis. If parents agree to provide their contact information to the medical investigators, they are informed of the and the inclusion criteria are checked. Thirty-four participants with MIH aged from 8 to 12 years were given medical and dental history, oral examination that included visual inspection with smooth-surfaced standard mouth mirror (#5) and tactile inspection with standard dental probe (PCP11), and radiological examination that included panaromic and/or periapical radiographs. Twenty-two subjects qualified and were registered to the study.

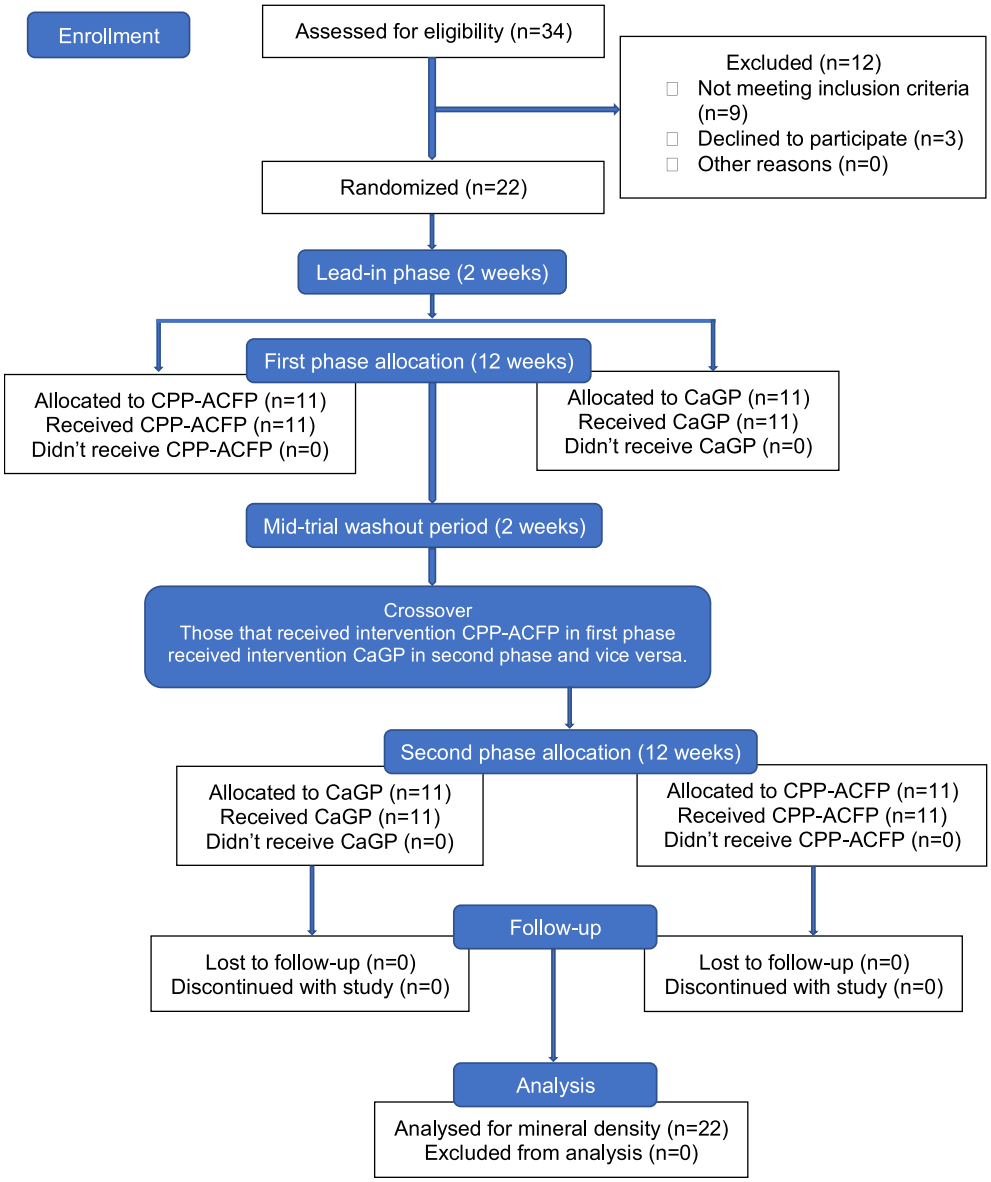


Fig. 1. Flow diagram detailing the stepwise methodology of this cross-over, randomized-controlled study design as first phase and second phase. The number of teeth in the diagram indicates the number of children's incisors affected by molar-incisor hypomineralization with white/creamy and yellow/brown demarcated opacities.

2.7. *Randomisation*

Participants meeting all eligibility requirements are allocated to one of the two study arms (MI Paste Plus™ or Medical Minerals Gel) in a 1:1 ratio using simple randomization with computer generated random numbers. Participants who used Medical Minerals Gel in first phase used MI Paste Plus™ in second phase and vice versa.

2.8. Blinding

All tubes, containing the interventions, were masked so the participants were unaware of the paste being used. Participants, dentists caring for participants, laboratory technicians and investigators assessing the outcomes will be blinded to group assignment.

2.9. Outcome measurements

Baseline and post-application evaluation and analysis were done using by LF [DIAGNOdent™ Pen (KaVo, Biberach, Germany)]. After the isolation of the teeth, DIAGNOdent™ Pen's A type probe was used to obtain quantitative values from hypomineralized lesions of permanent incisors affected by MIH. Before each reading, the device was calibrated on a ceramic block according to the manufacturer's instructions. After setting appropriate functions on the device, the probe A (for smooth surfaces) of the device lightly contacted enamel surfaces and moved until the maximum score was obtained. The probe was steered through the entire surface, and the highest value on the screen was recorded. This peak value was documented at each measurement point. For the reliability of the measurements, a training was carried out for two weeks before study, using the device on different lesions. Also control measurements were performed on an unaffected tooth and then healthy area of the affected tooth in children. Then, three readings from mesial, distal, and middle of lesion were taken from each hypomineralized area and the mean of these values was recorded. In the first and third months of using agents, the readings for the same teeth and lesions were taken using LF. MIH diagnosis criteria approved by the EAPD was used for the classification of the lesions [19]. Accordingly, demarcated opacities are divided into two separate subtypes as white/creamy and yellow/brown. LF of smooth enamel surfaces with white/creamy and yellow/brown opacities larger than two-millimeter diameter was measured.

2.10. Statistical analysis

Descriptives statistics were given as; mean ± standard deviation (SD) for continious random variables and frequency with percentage for discrete random variables. To compare before and after LF values in agents, the paired *t*-test was used. Chi-square test was used to compare the categorized variable for the percent of change before and after application on LF values, between agents. For all statistical analyses SPSS software version 22.0 (SPSS Inc, Chicago, IL, USA) was used, and significance level for the statistical tests was considered as $p < 0.05$.

3. Results

A total of twenty-two children [13 (59.1%) girls and 9 (40.9%) boys] ages between 8 and 12 years (mean ± SD: 9.23 ± 1.41) with 167 permanent incisors were included in the study. The number of incisors with white/creamy or yellow/brown demarcated opacity affected by MIH in the children included in the study was 7.59 ± 0.67. Incisors evaluated according to different clinically diagnosed levels of MIH as white/creamy [$n = 115$ (68.9%)] or yellor/brown [$n = 52$ (31.1%)] demarcated opacities randomly assigned into two application phase.

There was statistically significant difference in LF values between baseline and post-application in both groups. Baseline and post-application LF values of MD is demonstrated in Table 1.

Table 1
Laser fluorescence values in the two study groups baseline and post-application

Lesion type	Agents (N)	Baseline†	Post-application†	Difference (95% Cl)††	*p*-value
White/creamy	CaGP (61)	7.82 ± 4.05	6.83 ± 2.86	0.99 (0.13615-1.85336)	0.024*
	CPP-ACFP (54)	6.87 ± 3.63	5.87 ± 2.84	1.00 (0.21411-1.77366)	0.013*
Yellow/brown	CaGP (25)	29.61 ± 6.28	24.44 ± 5.57	5.17 (2.82098-7.52622)	<0.001**
	CPP-ACFP (27)	34.30 ± 6.95	29.26 ± 7.98	5.04 (0.50328-9.59746)	0.031*

N: number; CaGP: calcium glycerophosphate; CPP-ACFP: casein phosphopeptide amorphous calcium fluoride phosphate; CI: confidence interval. †Values are shown as mean ± standard deviation; ††Difference defines the change between the post-application and baseline laser fluorescence values; *$p < 0.05$; **$p < 0.001$.

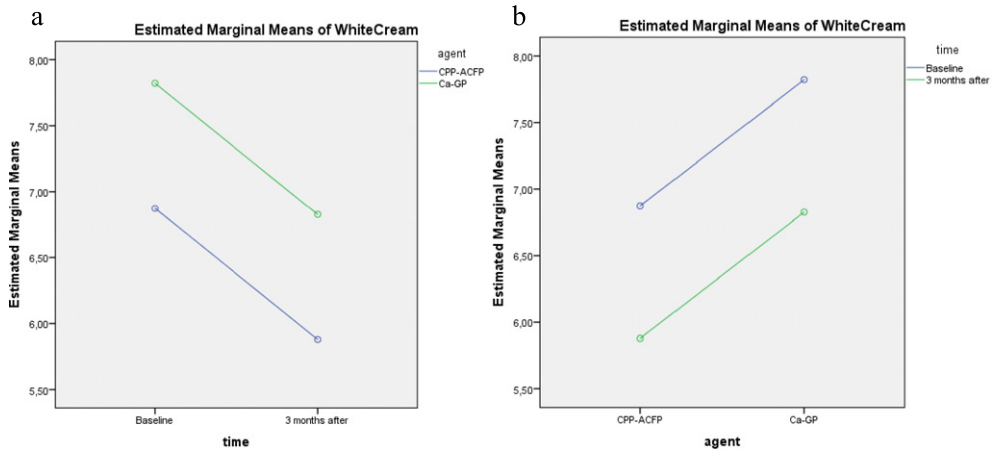


Fig. 2. a,b. The reduction in LF values suggests an increasing MD with CPP-ACFP and CaGP for white/creamy demarcated opacities. The numbers on the left vertical line represent the numerical values on the screen of the DIAGNOdent™ Pen device, which works with the LF principle. LF: laser fluorescence; MD: mineral density; CPP-ACFP: casein phosphopeptide amorphous calcium fluoride phosphate; CaGP: calcium glycerophosphate.

The reduction in LF values suggests an increase MD with CPP-ACFP and CaGP for white/creamy and yellow/brown demarcated opacities. For both groups, the rates of increasing MD are shown in Figs. 2a,b; 3a,b.

If the difference between the remineralizing agents' formulations for anyone measuring system was not considered clinically appropriate, non-inferiority/equivalence was defined, and the threshold was set at 20%. The difference between CPP-ACFP and CaGP according to after categorization of 20% increasing in MD in the percent of change before and after application on LF values; was not found statistically significant in white/creamy ($p = 0.970$) and yellow/brown ($p = 0.948$) opacities (Table 2).

There was no significant difference between the CPP-ACFP/CaGP and CaGP/CPP-ACFP applications regimens. The effect of the first application phase have disappeared completely before the beginning of the second phase.

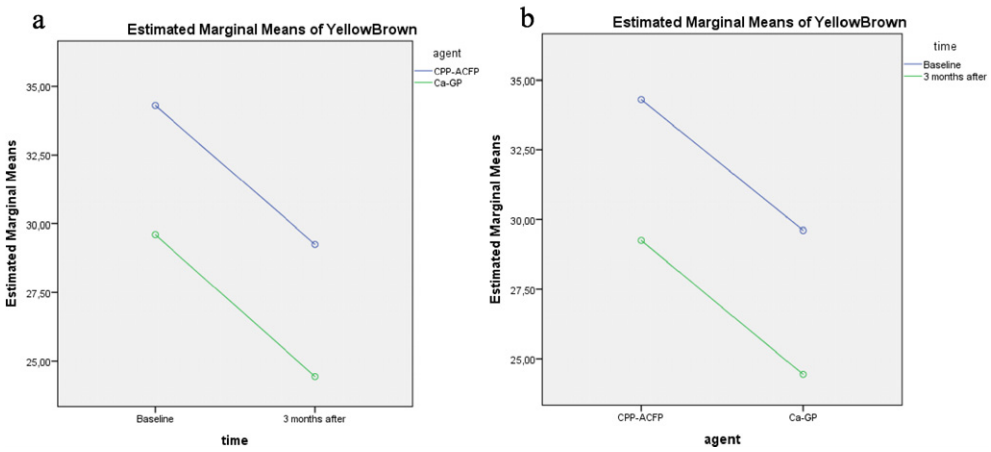


Fig. 3. a,b. The reduction in LF values suggests an increasing MD with CPP-ACFP and CaGP for yellow/brown demarcated opacities. The numbers on the left vertical line represent the numerical values on the screen of the DIAGNOdent™ Pen device, which works with the LF principle. LF: laser fluorescence; MD: mineral density; CPP-ACFP: casein phosphopeptide amorphous calcium fluoride phosphate; CaGP: calcium glycerophosphate.

Table 2
Number of change in laser fluorescence values corresponding to CPP-ACFP and CaGP for white/creamy and yellow/brown lesions

Lesion type	Change before and after application on LF values	CPP-ACFP	CaGP	*p*-value
White/creamy	< −20%	21 (45.7%)	25 (54.3%)	0.970
	≥−20%	33 (47.8%)	36 (52.2%)	
Yellow/brown	< −20%	11 (55%)	9 (45%)	0.948
	≥−20%	16 (50%)	16 (50%)	

LF: laser fluorescence; CaGP: calcium glycerophosphate; CPP-ACFP: casein phosphopeptide amorphous calcium fluoride phosphate.

4. Discussion

This cross-over RCT study showed that the use of MI Paste Plus™ containing CPP-ACFP and Medical Minerals Gel containing CaGP in addition to standard fluoride-containing toothpaste for three months provides a statistically significant increasing in enamel MD in the white/creamy and yellow/brown demarcated opacities of permanent incisors affected by MIH.

Although fluoride interventions have the highest supporting evidence in the remineralization of teeth affected by MIH; additional preventive and remineralization agents are often required especially when the MD of enamel is extremely reduced such as MIH [1]. Among the MIH studies in the literature, the agents used to prevent demineralization, provide remineralization, increase MD and eliminate hypersensitivity are: fluoride varnishes [11,20–22], CPP-ACP [10,11,20,22], CPP-ACFP [10,22], arginine [23] and ozone [22].

CPP-ACP complex shows its effect in supersaturation of phosphate, calcium and fluoride ions in saliva to modulate bioavailability, remineralization of hydroxyapatite, prevention of demineralization of hydroxyapatite, bactericidal and bacteriostatic effects on microorganisms of dental biofilm, maintaining of ionic concentration under the demineralized enamel for remineralizing process and buffering of the dental biofilm by providing high ion levels [13,24]. CPP-ACP improves the remineralization process and increases MD as a complementary source of calcium and phosphate. Studies show that CPP-ACP is effective in the remineralization of enamel subsurface lesions [25,26]. CPP binds to calcium, phosphorus and fluoride ions and stabilizes them in the form of amorphous nanocomplexes. While fluoride-containing agents mainly remineralize the superficial layers of enamel, the CPP-ACP complex can achieve its aforementioned effects on the body of the lesion. Moreover, the synergistic effect of fluoride and CPP-ACP in remineralization has been proved to the formation of CPP-ACFP [10,27]. Bakkal et al. [10] investigated the effect of CPP-ACP and CPP-ACFP on teeth affected by MIH and LF findings showed that a statistically significant increasing of enamel MD in CPP-ACP and CPP-ACFP in 30 days of use. The findings of the present study showed that the three-month usage of MI Paste Plus™ containing CPP-ACFP provided statistically significant increasing in enamel MD in permanent incisors affected by MIH. These results support that the use of products containing CPP-ACFP is effective in the increasing in MD of teeth with MIH [10,22].

Calcium glycerophosphate, as organic polyphosphate, has the ability to be absorbed onto enamel surfaces through phosphate radicals. CaGP prevents demineralization, provides remineralization and increases MD by direct interaction with enamel, buffering of plaque pH, reduction of plaque amount, modification of plaque metabolism and increasing calcium and phosphorus levels in plaque [16,28]. Although there is no study about its use and effect in MIH, its remineralizing properties have been demonstrated in several studies. In a study by Zaze et al. [29], it was shown that 500 ppm fluoride-containing toothpastes with CaGP were as effective in remineralization as 1100 ppm fluoride-containing toothpastes. do Amaral et al. [30] showed that CaGP-added low fluoride toothpastes were as effective as standard higher fluoride toothpastes in preventing demineralization. Also, Rezende et al. [31] showed that toothpastes containing CaGP and more than 500 ppm fluoride were successful in protecting enamel from demineralization. Contreversely these studies findings, Carvalho et al. [32] stated that the addition of CaGP to fluoride varnishes had no effect on increasing mineral content. The present study showed that, regardless of the cross-over study design, Medical Minerals Gel including CaGP increased statistically significant MD in permanent incisors affected by MIH.

Limitations of our study mainly concerns the MIH representativeness of our sample. So, age of observed population, from 8 to 12 years, did not allow registration of initial stage on index teeth. Age of 8 years was recommended as optimal one for MIH examination [33]. In the same, participants' daily oral hygiene procedures with standard fluoride-containing toothpaste may also influence the observed mineral increase. Although the importance of color and opacities of a hypomineralization was recognized in this study, the distribution of size according to color and location was not presented. At least, the severity of MIH presence in our trial was not associated with the number of teeth per child in a context where only the MIH-affected incisors were retained at the expense of the molars. Advanced stages of hypomineralization were more susceptible in patients with a high number of affected teeth [19].

5. Conclusions

The reduction in mineral content observed in enamel affected by MIH can be effective by using different remineralizing agents. In addition to oral hygiene practices with standard fluoride-containing toothpaste, CaGP or CPP-ACFP-containing products can be used to increase enamel MD in MIH. The use of remineralization agents had an effect regardless of the cross-over study design. This situation supported the use of various remineralization agents in developmental enamel defects such as MIH, regardless of the order of use. Studies on protective and remineralizing approaches and agents for the mineral content of enamel in hypomineralized teeth should be conducted to include larger sample size with longer term to obtain more clinically useful findings.

Conflict of interest

The authors declare that they have no conflict of interest.

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