Effect of Remineralization Agents on Molar-Incisor Hypomineralization-Affected Incisors: A Randomized Controlled Clinical Trial

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Objective: *The objective of this study was to evaluate the remineralization effect of two different mineral containing agents on white/creamy and yellow/brown demarcated opacities in incisors in children with molar-incisor hypomineralization (MIH) by using laser fluoresence (LF).* ***Study Design:*** *Fifty-three children (n=401 lesions) with MIH were randomly divided into three groups: (1)calcium glycerophosphate (CaGP), (2)casein phosphopeptide amorphous calcium fluoride phosphate (CPP-ACFP) and, (3)control (1450 ppm fluoride toothpaste). Remineralization was evaluated by means of LF, at baseline, after one and three-months. Anova Test for Repeated Measurements in intra-group comparisons in evaluating the effectiveness of remineralization agents. One-way Variance Analysis (ANOVA) and Tukey-Kramer Multiple Comparison test were used in the comparisons between groups and, Student Newman Keuls Multpile Comparison Test was used to determine the differences between the measurement averages in case of p<0.05.* ***Results:*** *There was a significant improvement in MIH-lesions over time in all groups (p<0.001), with no differences between groups. The highest percentage of change was observed in CPP-ACFP in lesions LF≤20 scores and the mean percentage of change LF>20 scores, the highest percentage changes in CaGP. There was no significant difference between the groups over the time for all the used outcome measures (p>0.05).* ***Conclusion:*** *The additional use of both mineral containing agents in MIH-affected teeth improved these hypomineralized lesions with mineral deposition. Even if both agents could be used in the hypomineralized teeth with demarcated opacities, future studies are recommended the long-term effect of these mineral containing agents with longer observation and a larger sample size.*

Keywords: *Molar incisor hypomineralization, Calcium glycerophosphate, Casein phosphopeptide-amorphous calcium phosphate nanocomplex, Tooth remineralization.*

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INTRODUCTION

Molar-incisor hypomineralization (MIH) is characterized by qualitative developmental defects of enamel of one or more first permanent molars (FPMs), with or without involvement of incisors[1] and different phenotypes of MIH affect different teeth to varying severity.[2]

MIH-affected enamel shows increased amounts of proteins like serum albumin, type-I collagen, ameloblastin, alpha-I-antitrypsin and antithrombin-III, which were found to inhibit the growth of hydroxyapatite (HA) crystals and enzymatic activity during enamel maturation, resulting in an overall reduction of minerals and also has been shown to have increased porosity and disorganized prisms with loss of distinct boundaries between rods.[3] Concentration of the main chemical composition, primarily calcium (Ca) and phosphorus (P), is significantly reduced in hypomineralized enamel compared to healthy enamel.

Laser fluorescence (LF) most likely detects some fluorophores that are organic in nature. Farah *et al* showed a moderately strong correlation between the mechanical properties of MIH-enamel and LF scores.[4]

Minimally invasive treatment approaches adopt a philosophy that integrates prevention, remineralization and minimal intervention in the treatment of teeth affected by MIH.[5,6] Consequently, remineralization agents are often recommended for the management of MIH in order to increase the mineral content of the hypomineralized areas although scientific evidence of the effectiveness of this treatment is still limited.[7]

Fluoride (F) has still remained gold standard as remineralization agent considering more than 50 years of clinical success. Calcium fluoride serves as a source of F for the formation of fluoroapatite, thereby inhibiting demineralization and enhancing remineralization.[8] Casein phosphopeptide amorphous calcium phosphate (CPP-ACP) complex is an appealing delivery system for bio-available Ca and phosphate (PO_4) ions, maintaining an amorphous state of supersaturation with respect to enamel, decreasing demineralization and promoting remineralization.[9,10] In previous researches which were set out to determine the effect of remineralizing agents in MIH have reported that F-containing and non-fluoridated CPP-ACP derived agents both strengthened and remineralized enamel and reduced the porous structure at depth of lesion and affected surfaces of MIH.[6,9-11] Studies since the 1930s have reported the importance of using Ca and PO_4 derivatives for favouring the remineralization process in dental caries.[12] Calcium glycerophosphate (CaGP), which is used medically as a source of Ca and PO_4, has demonstrated anticariogenic properties.[12,13] CaGP is an organic PO_4 capable of adsorption to enamel surfaces, leading to the release of Ca ions, which act on the remineralization process.[14-16] The results of demineralization tests confirm that it exerts a powerful protective action on surface of the enamel. This effect was independent from the action of F.[15] CaGP shows a protective effect by increasing the resistance of HA. Its effect in biofilms may be related to the enhance of Ca and PO_4 levels, buffering capacity and reduced the mass of the biofilms.[14] It is thought that the effectiveness of caries prevention by increasing cumulative effect with F.[15,16]

Today, different detection and evaluation methods are used in remineralization researches. In the detection of demineralization and hypomineralization, it is very important to determine the activity, depth, and other characteristics of the lesion.[17] Researchers have shown an increasing interest in non-destructive methods for the assessment and longitudinal monitoring of mineral changes in enamel, such as LF. DIAGNOdent™ is the application of LF method in dentistry, which is based on the principle of absorbing and scattering the laser beam at a different rate compared to the healthy structure with the demineralized/hypomineralized areas.[18]

In current literature there is a lack of evidence for the effectiveness of remineralization agents to the MIH-affected teeth. The objective of this study was to evaluate the remineralization effect of two different mineral containing agents (CaGP and CPP-ACFP) on white/creamy and yellow/brown demarcated hypomineralized opacities in incisors with MIH *in-vivo*, to be used in addition to routine oral hygiene using LF. The hypothesis adopted in the current study is that the *in-vivo* use of commercially available remineralization agents, in addition to routine oral hygiene, does have an effect on and assess of remineralization of demarcated hypomineralized opacities over time by LF.

MATERIALS AND METHOD

The study was approved by the Clinical Researches Ethical Committee of the School of Dentistry, Marmara University under the protocol number 2019-297, and was conducted in accordance with the principles of medical research involving human subjects described by the Declaration of Helsinki. Remineralization of demarcated hypomineralized opacities in incisors with MIH was assessed during three months using by LF.

This trial was performed as a prospective, randomized, controlled trial. The allocation of subjects followed a randomization scheme with stratification for gender. No changes to the original protocol were made during or after the trial.

The subjects enrolled in the study were children with MIH who applied to the Department of Pediatric Dentistry, School of Dentistry, Marmara University. Informations about the study were explained to the parents and then the consent forms were signed.

This single-centre trial took from January 2019 to May 2019.

Sample Power Calculation

The prospective sample power calculation was done using G*Power 3.1 ANOVA for repeated measures, between factors (Franz Faul, Germany). To assess the influence of CPP-ACFP on the reduction of MIH lesions , a power analysis was conducted as described by Beerens *et al* [19] found a statistically significant, but clinically irrelevant, natural reduction in fluorescence loss, of 0.9 per cent (SD=0.9 per cent), during a 12-week time period. Finally, the software computed the minimum sample size of 50 for two experimental groups and control at significance of p-value<0.05 with actual power quantified as 0.81 a priori protocol for tests sample power analysis.

Inclusion Criteria

All participants fulfilled the following requirements:

- Children girls or boys between eight to 12 years without any systemic disease and/or syndrome/anomaly/allergy were included in the study.
- MIH diagnosis criteria published by Ghanim *et al* [20] in 2015 were used in diagnosis and evaluation.
- Presence of MIH was defined as present whenever any of the FPMs exhibited demarcated hypomineralized areas, post-eruptive enamel breakdown, atypical caries or atypical restorations.
- The selection criteria for maxillary and mandibular central and lateral incisors were: fully erupted, with white/creamy or yellow/brown demarcated opacity, no cavitated caries and/or post-eruptive enamel breakdown, no previous preventive or restorative treatment of lesions.
- No proven or suspected milk protein allergy and/or sensitivity, or allergy to benzoate preservatives, as both are components of the CPP-ACFP products.

Exclusion Criteria

- Patients who show no cooperation in using remineralization agents.

- Patients with systemic disease and/or syndrome/anomaly/allergy.
- Teeth with post-eruptive enamel breakdown, atypical caries or atypical restorations.
- Children who have hypersensitivity due to MIH.
- Loss of tooth structure because of caries or trauma, fillings and/or with other developmental enamel defects such as fluorosis, amelogenesis imperfecta etc.
- Proven or suspected milk protein allergy and/or sensitivity, or allergy to benzoate preservatives.

Randomization and Intervention Procedures

Participants, complying with the inclusion criteria as determined by one trained pediatric dentist (BS), were randomly assigned by BS to group the CaGP, CPP-ACFP or the control group, as determined by a computer-randomization scheme, created and locked before the start of the study. Participant allocation was kept separate from the data recording files in a locked closet. Data were collected and coded based on participants' ID number and a sequential number in order of date of study visits. Data analysis was performed blind for group allocation.

Participants received (1) CaGP (R.O.C.S.® Medical Minerals Gel includes CaGP, magnesium and xylitol, R.O.C.S. Trading GmbH, Munich, Germany) (27 children), (2) CPP-ACFP (10% CPP-ACP plus 0.2% NaF; GC MI Paste Plus™, GC Europe) (16 children), (3) Control (routine dental home care with Colgate Toothpaste including 1450 ppm F, Colgate Oral Pharmaceuticals, New York, NY) (ten children). (shown in Figure 1)

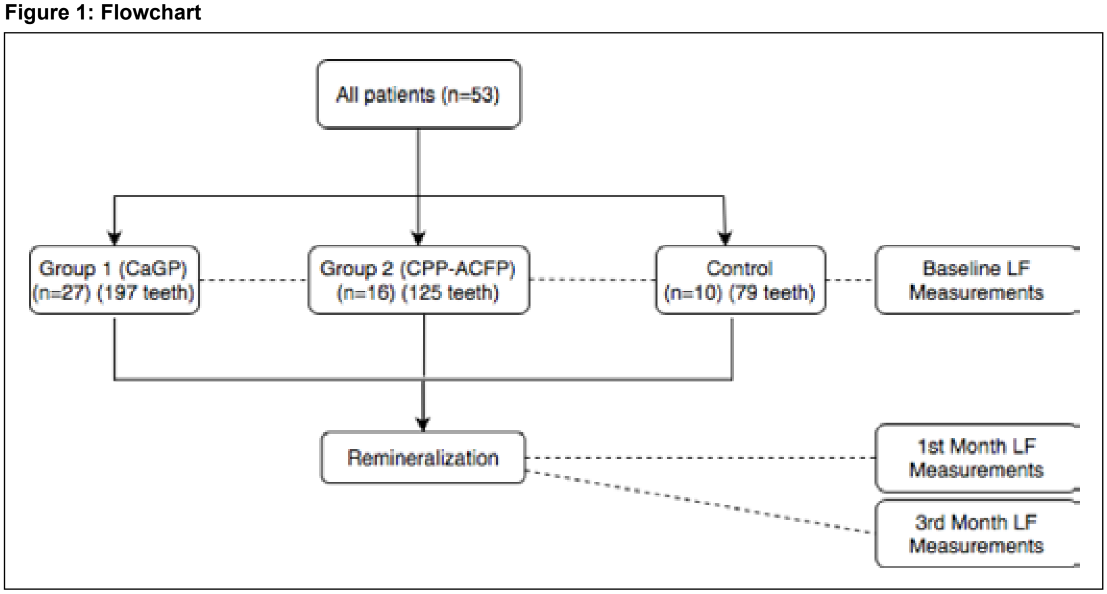
All patients received verbal and written instructions with respect routine oral hygiene procedures and product usage by researcher. They were advised how to brush properly using a fluoridated dentifrice (Colgate Total with 1450 ppm F) (i.e. at least twice a day, either with a hand toothbrush or an electric toothbrush for at least two minutes). In addition, participants were instructed to use their respective paste twice a day once in the morning after brushing and once in the evening immediately before going to bed. They should leave it on their teeth to slowly dissolve overnight. Children were asked to apply with a clean finger to the tooth surfaces in accordance with the manufacturer's instructions, under the control of their parents. Parents were informed to apply at least a pea-size amount to the tooth surfaces in each arch using a clean, dry finger and keep the study product in the mouth for as long as possible. Participants were instructed not to rinse, eat or drink for minimum 30 minutes afterwards.[11] Compliance of the patients were checked by asking questions regarding product usage at each visit. Furthermore, parents were asked to bring the study product to each visit. The parents were informed not to administer additional F during this investigation. They were further asked to contact the study investigator if restorations were made.

LF Measurements

Remineralization was evaluated by means of LF. The red diode laser beam with a 655 nm wavelength passes through the fiber bundle and carries to the tooth surface with a special tip in the device. Demineralization in the enamel reduces the fluorescence of the tooth. The greater the beam intensity reflected back from the tooth, the greater the depth of the lesion.[21] It has been suggested that the reason for the increased scores in hypomineralized enamel may be due to the increased protein ratio in the enamel and/or the scattering of incoming rays by nonhomogeneus enamel.[4]

After the isolation of the teeth, LF device's (DIAGNOdent™ Pen, KaVo, Biberach, Germany) A type probe was used to obtain quantitative scores from hypomineralized lesions. 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 score on the screen was recorded. This peak score 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 opacities. Also control measurements were performed on an unaffected tooth and then healthy area of the affected tooth in children. Then, three scores from mesial, distal, and middle of demarcated area of tooth were taken from each

Figure 1: Flowchart



hypomineralized lesion and the mean of these scores was recorded. In the first and third months of using remineralization agents, the scores for the same teeth and lesions were taken using DIAGNOdent™ Pen. Depth of defects were subjected using LF threshold. The LF threshold was set between five and 20.[22]

Statistical Analysis

All statistical analyzes were performed by using Statistical Package for Social Sciences software (SPSS 22 for Windows, SPSS Inc., Chicago, Illinois, USA). Descriptive values of the data obtained were calculated as mean±SD. Anova Test for Repeated Measurements in intra-group comparisons in evaluating the effectiveness of remineralization agents. Student Newman Keuls Multiple Comparison Test was used to determine the differences between the measurement averages in case of $p<0.05$. Also, One Way Variance Analysis (ANOVA) and Tukey-Kramer Multpile Comparison test were used in the comparisons between groups. Test was used to determine the differences between the measurement averages in case of $p<0.05$.

RESULTS

From the 102 screened children with MIH; a total of 53 children [28 (53%) girls and 25 (47%) boys] ages eight to 12 years (9.34±1.4) with 401 demarcated hypomineralized teeth were evaluated according to different clinically diagnosed levels of MIH as white/creamy and yellow/brown demarcated lesions >one-mm in diameter but noncavitated teeth randomly assigned into three groups: 27 children with 197 teeth in Group 1 (CaGP), 16 children with 125 teeth in Group 2 (CPP-ACFP) and ten children with 79 teeth in Group 3 (F toothpaste, control) were evaluated by LF.

Lesion Changes Assessed by LF

At baseline for LF scores, no significant differences between groups were found ($p>0.05$).

Repeated-measures ANOVA showed significant changes in the LF scores in time or between the groups ($p<0.001$).

Data concerning LF scores at baseline scored less than 20 or greater than 20, remineralization was achieved over three months period and significant improvement in LF scores was observed in both experimental groups ($p<0.05$). A similar trend was seen in the control group, LF measurement that scored less than 20; remineralization was observed over time and changes were found significant ($p<0.001$), although no significant changes in lesions that scored greater than 20 over time ($p>0.05$) (Table 1).

Table 1: Comparisons of Mean±SD of LF Over Studied Time

	Baseline Mean±SD	1st month Mean±SD	3rd month Mean±SD	p
≤20				
CaGP	**8.10**±5.00	**7.53**±4.98	**6.99**±5.02	**0.005**
CPP-ACFP	**8.19**±5.71	**7.07**±4.91	**6.43**±4.41	**0.025**
Control	**6.13**±2.09	**4.94**±3.14	**5.37**±2.87	**0.000**
>20				
CaGP	**36.66**±19.41	**30.30**±15.95	**25.20**±14.03	**0.004**
CPP-ACFP	**38.89**±21.28	**32.82**±21.94	**32.56**±22.81	**0.043**
Control	**24.39**±12.80	**24.08**±10.90	**20.75**±10.60	**0.22**

The highest percentage of change between the baseline and in time was observed in CPP-ACFP, followed by control and CaGP in lesions that scored less than 20. The mean percentage of change LF scores greater than 20, the highest percentage changes in CaGP, followed by CPP-ACFP and control, respectively (Table 2).

Table 2: Mean of Percentage Change of LF Over Studied Time

	Mean Difference	p
≤20		
CPP-ACFP–CaGP	**4.91**	**0.087**
Control–CaGP	**4.29**	**0.250**
CPP-ACFP–Control	**0.62**	**0.854**
>20		
CaGP–CPP-ACFP	**14.26**	**0.004**
CaGP–Control	**25.68**	**0.001**
CPP-ACFP–Control	**11.42**	**0.253**

When the mean differences were compared, it was seen that there was no statistically significant difference between the groups in lesions that scored less than 20. When the mean of the percent change in lesions that scored greater than 20 between baseline and third months is compared, the difference between CaGP and CPP-ACFP ($p<0.05$) and CaGP and control is found statistically significant ($p<0.01$) while the difference between CPP-ACFP and control is not found statistically significant ($p>0.05$) (Table 3).

Table 3: Intergroup Comparisons of Mean of Percentage Changes in LF Scores

	Number of Measurements	Mean of Percentage Change (%)
≤20		
CaGP	**453**	**-5.31**
CPP-ACFP	**387**	**-10.22**
Control	**201**	**-9.60**
>20		
CaGP	**138**	**-25.59**
CPP-ACFP	**123**	**-11.33**
Control	**36**	**0.09**

Adverse Effects

There were no adverse effects or harms experinced by the participants influencing general health of the participants, for either group.

DISCUSSION

Preventive dentistry aims to prevent initial carious lesions known as white spot lesions, developmental enamel defects, and hypomineralized areas without cavitation.[23]

Many different treatment approaches can be applied on incisors affected by MIH. The priority in the treatment of non-cavitated hypomineralized areas is remineralization protocols within the scope of minimally invasive and preventive dentistry.[24] Although it is known that the application of high concentrations of F provides a remineralization in the superficial layer of demineralized enamel, this effect is not been observed in deeper layers. For this reason, it is recommended to apply lower concentrations of F, which allows the absorbtion of Ca and F ions from saliva. In addition, researches on alternative remineralization agents to F are becoming common.[25]

Improving the mineralization of MIH-teeth after eruption is possible according to some *in-vivo* and *in-vitro* studies; though, a complete resolution seems to be difficult due to the depth and/or the thickness of these lesions. In an effort to remineralize the MIH-teeth, the long-term use of products containing CPP-ACP is recommended especially at early stages where the surface enamel of newly erupted teeth is not completely matured.[11,26]

Some authors suggest that CPP-ACP may accelerate and increase the potential maturation of MIH-enamel structure, improving its mechanical properties after tooth eruption. Decreasing the surface porosity may reduce the caries risk and sensitivity due to thermal/tactile stimulation.[6,27] In a study conducted by Baroni and Marchionni, it was hypothesized that the application of CPP did not alter the surface morphological characteristics of MIH-molars *in-vivo.*[6] The results suggested that the use of a mixture of Ca and PO_4 ions carried by large molecules can be indicated in MIH-teeth where such minerals are deficient. CPP-ACP supplementation accelerates and increases the potential maturation of MIH-enamel structure, which may or may not take place over a much longer time. In conclusion, since improvements were found after the application of Ca-PO_4 casein on MIH-molars, the results of the present study support the rejection of the tested hypothesis, warranting investigations of new products containing fluoridated CPP-ACP, and needing adjustments of administration protocols. Given the absolute lack of *in-vivo* studies and supplementation trials, protocols with CPP-ACP may help functional restorative techniques in treated MIH-molars and aesthetics in untreated incisors.[6] However, there is a lack of evidence as regards the long-term effectiveness and protocols of products containing CPP-ACP in MIH-lesions.

Restrepo *et al*. observed no favorable effect on the remineralization of MIH-lesions in anterior teeth after four applications of F varnish.[7] However in our study, it was observed that the use of fluoridated toothpaste provided remineralization in incisors affected by MIH.

Although the efficacy of CPP-ACFP for the prevention and regression of incipient lesions has been demonstrated *in-vitro*[28,29] there is no reliable evidence for the treatment of MIH-lesions *in-vivo* and the long-term effect of this remineralizing agent is unclear.

The F ions contained in CPP-ACFP might slowed the penetration of the Ca and PO_4 ions to the inner layers of the lesions and caused some early precipitation of these ions in the outer enamel surfaces.[30] In parallel with this information, it was determined that higher levels of remineralization in the CPP-ACFP group in LF scores less than 20, in our study.

In a study conducted by Bakkal *et al*, it was observed that CPP-ACP and CPP-ACFP were found to have achieved a significant decrease in LF scores in one-month.[11] This pilot study showed that using CPP-ACP and CPP-ACFP had a positive effect in reducing hypomineralization on enamel surfaces of MIH-teeth for one-month period.[11] Similarly, results of our study showed that CPP-ACFP could be an effective remineralization agent in incisors affected by MIH in the short treatment period.

CaGP is an organic polyphosphate also has the ability to adsorb to enamel surfaces through the PO_4 radicals of its molecule. CaGP, however, acts at different levels of the demineralization process.[31] When this organic PO_4 adsorbs to enamel surfaces, virtually all PO_4 groups are neutralized by Ca and hydroxyl groups of HA molecules, so that Ca ions from CaGP are released next to enamel surfaces.[31]

Research on CaGP and sodium monofluorophosphate interactions show that the ratio of monofluorophosphate to CaGP affects the demineralization of HA and enamel. It is known that use of sodium monofluorophosphate and CaGP separately is significantly less effective than a combination of both. Regardless of the presence and mechanism of F, CaGP has a strong protective effect.[31] In a study conducted by Grenby and Bull, pre-treating HA discs with NaF conferred a relatively long-lasting protective effect against demineralization.[15] According to this study, CaGP and F do not share the same mechanism of action, but their effects can be cumulative.[15] It has been stated that the CaGP interacted directly with the outer layers of the HA. Many possible mechanisms for the cariostatic properties of CaGP have been proposed. These include direct interaction with enamel, plaque pH-buffering, reduction in plaque mass, modified plaque metabolism, and elevation of Ca and PO_4 levels in plaque.[31] CaGP alone has cariostatic properties and has repeatedly demonstrated anti-caries properties *in-vivo*. It would seem that these effects can be achieved readily but only if CaGP is applied frequently and at relatively high concentrations.[31] Further, F and CaGP have an additive effect and probably influence dissolution of enamel via different mechanisms. The potential anti-caries benefits of buffering of plaque acid are self-evident.[31]

The low-F dentifrice with 0.25% CaGP demonstrated efficacy similar to that of the positive control (1,00 dentifrice) with respect to *in-situ* demineralization.[32] The results of a study about fluoridated toothpastes indicate that clinical efficacy of low-F toothpaste supplemented with the addition of CaGP leads to similar efficacy when compared to 1100 ppm F.[33] Zaze *et al* showed that low-F toothpaste (500 µg F/g) maintains the same efficacy as that of a 1100-µg F/g toothpaste when supplemented with 0.25 % CaGP and provides the best effect against *in-vitro* enamel demineralization.[16] In a study conducted by Rezende *et al*, remineralization of different F solutions on the enamel surface in the presence of CaGP (0.13%) was evaluated.[34] Although there was no statistically significant difference between groups with different levels of F containing agents, it was reported that agents containing CaGP increased remineralization significantly compared to the control group.[34] In parallel with these study results, in our study, it was observed that the hypomineralized areas on incisors affected by MIH was remineralized with CaGP. This study was the first to address these aspects.

In teeth with LF scores greater than 20, it was observed that CaGP performed statistically significantly more remineralization compared to CPP-ACFP and control group in comparing the mean values of intergroup change at the end of the three-month period ($p<0.01$). This can be explained by the CaGP due to the affinity of the enamel to the HA structure by exceeding the superficial remineralization layer made by F.[31] Despite the promising results reported for CaGP-containing toothpastes, no clinical evidence is still available to attest the anticaries effects with PO_4 salts on the progression of caries lesions.

The hypomineralized teeth with high LF scores tend to have low mechanical properties of MIH-enamel suggest lower mineral content and the increased LF scores suggest increased organic content. The altered prismatic structure and the inhomogenity of hypomineralized enamel may have resulted in or contributed to the increased LF scores. Another factor that may contribute to the increased LF from MIH-enamel is its darker colour. In this study the higher LF scores recorded in MIH-lesions indicated a lower mineral content at baseline. High LF scores in non-carious teeth only indicate hypomineralized enamel. The increased scores in hypomineralised enamel may be related to proteins in the hypomineralized enamel and/or light scattering by the inhomogeneous enamel. Bacterial metabolites may not be the only fluorophore resulting in an increase in LF scores, especially in hypomineralized enamel. Regardless of the nature of the fluorophores in MIH-enamel, however, increased LF score indicates defective enamel.[4] Assuming that the increase in LF scores represents a real increase in organic substance across the all scale of the scores, it seems that beyond LF scores of around 20, the mechanical features are no longer that susceptible to the quantity of organic substance in hypomineralized enamel.[4]

As a result, we can say that the application of CaGP and CPP-ACFP for three-months provides remineralization in incisors affected by MIH. Future investigations may increase the experimental time to determine effectiveness of remineralizing agents over time.

Strength and Limitations of this Study

The study was performed in a group of children in Istanbul, Turkey. Turkey is part of Eastern Europe and has no water fluoridation. Therefore, water fluoridation did not affect the outcome of this study. Many different treatment approaches can be applied on incisors affected by MIH. The priority in the treatment of non-cavitated hypomineralized areas is remineralizing protocols within the scope of minimally invasive and preventive dentistry. The findings of this study are applicable to MIH. On the other hand, this study has some limitations, as limited number of subjects and a short-term application period. In addition, it is not possible to know that patients were using the products correctly at home due to the *in-vivo* study. As for all randomized clinical trials, non-compliance of the subject could have influenced the result. We did not use an application tray, saliva could now also influence possible remineralization. One of the possible limitations influencing the results of the study is the question can also be raised if there was a similarity of intervention. As there might be a taste difference between the two products. Other possible limitation is that the control group did not apply a placebo gel.

CONCLUSIONS

The use of CPP-ACFP and CaGP containing remineralization agents in children with MIH lesions improved these lesions over 3 months period of the study, as evaluated by LF measurements. Based on data obtained from our study, it was concluded that both CPP-ACFP and CaGP containing agents showed remineralization potential on hypomineralized enamel on incisors affected MIH by using LF. This investigation provided additional information for better understanding of the mechanisms of mineral containing agents, therefore being possible to accept the study's hypothesis.

It may be advisable to conduct researches with different remineralizing agents and LF method, longer follow-up and more sample size to support these findings. Thus, clinical application of these agents will be a novel noninvasive patient-friendly and effective method to treat reversible carious lesions. However, further *in-vivo* studies are required to reassure the superiority of CaGP and CPP–ACFP. Further research may help assess that LF readings may indicate for evaluation of MIH tooth and also mineralising agents.

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