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Clinical Evaluation of Remineralizing Agents in Children with Hypomineralized First Permanent Molars

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ABSTRACT

Pediatric dentistry now requires clinical assessment of remineralising drugs for children with hypomineralized first permanent molars, especially because to the increased frequency of molar incisor hypomineralization. First permanent molars with low mineral content are more susceptible to caries, sensitive, and mechanically compromised, which can harm a child's dental health and quality of life. Remineralization with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), fluoride varnishes, calcium glycerophosphate (CaGP), and other calcium phosphate-based compounds can repair molar incisor hypomineralization (MIH) related enamel defects without surgery. These remineralising agents will be clinically tested to improve the mineral content and structural integrity of hypomineralized first permanent molars in children. In-situ and in-vivo investigations assessed the effectiveness of remineralization therapies over time. Both fluoride-based and calcium phosphate formulations improved enamel remineralization, with CPP-ACP and CaGP showing excellent outcomes in mineral content and hypersensitivity. These medicines also reduced lesion size and severity, suggesting they might treat MIH abnormalities. However, the study stressed the need for longer follow-ups and bigger sample numbers to further understand these remineralising drugs' long-term efficacy and therapeutic application techniques. These results suggest that remineralising drugs, especially calcium phosphate-containing ones, may be a potential non-invasive therapy for hypomineralized first permanent molars.

Keywords: *Calcium Phosphate, Children, Hypomineralized, Phosphopeptide-Amorphous, Remineralizing Agents*

1. INTRODUCTION

One of the most serious issues that pediatric dentistry must contend with is the occurrence of dental enamel hypomineralization, which is especially prevalent in the case of first permanent molars [1]. Children whose teeth are affected by hypomineralized first permanent molars typically experience greater dental sensitivity, an increased chance of developing caries, and difficulties during restorative operations as a result of the quality of their enamel being damaged. It is common for these symptoms to be brought on by a decline in the quality of the enamel. One particular form of this illness is known as Molar Incisor Hypomineralization (MIH), and it is characterised by a shift in the mineral composition of enamel [2]. This condition is typically accompanied by alterations that are analogous to those that occur in permanent incisors. The number of instances with MIH continues to climb all over the world, and as a result, there has been an increase in the need for alternative treatment options that are both non-invasive and minimally invasive [3].

Remineralising agents are a particularly attractive method for treating this issue since they have the ability to increase performance. This makes them a very fascinating technology. Restoring the mineral composition of the enamel that has been destroyed, improving the structural integrity of the enamel, and reducing the sensitivity that is associated with MIH are the specific goals that these chemicals are designed to accomplish [4]. The purpose of this clinical evaluation is to explore a variety of remineralizing agents that are utilized in pediatric dentistry and to evaluate the effectiveness of these agents in improving the state of hypomineralized families of pediatric patients.

Understanding Hypomineralization and Its Impact

Hypomineralization: A pathophysiological perspective with regards to the process of enamel hypomineralization takes place during the mineralization phase of the amelogenesis formation process. The occurrence of this phenomenon takes place when there are disruptions in the process of mineralization, which ultimately leads to a reduction in the mineral content of the enamel matrix [5]. As a result of this, the enamel that is generated is delicate and porous, which makes it susceptible to deterioration after the eruption has occurred.MIH can be caused by a number of medical disorders, including systemic causes such as childhood illnesses, exposure to environmental toxins, and extended use of antibiotics [6]. These conditions can also be caused by a range of other conditions. Additionally, because of the porous nature of the enamel, bacterial penetration is made simpler, which leads to an increase in sensitivity as well as an increased likelihood of dental caries [7].

Characteristics of Hypomineralized Molars in Clinical Practice: First permanent molars that have been hypomineralized have enamel surfaces that contain opacities that range from yellowish-brown to creamy-white in colour [8]. In clinical procedures, including as brushing or restorative treatments, these teeth commonly demonstrate heightened sensitivity to thermal, chemical, or mechanical stimuli [9]. As a result, these operations can be painfully unpleasant and difficult to complete. Additionally, the insufficient binding strength that exists between restorative materials and hypomineralized enamel provides additional challenges for the administration of treatment in the dental office [10].

Remineralizing Agents: Types and Mechanisms

CaGP, or calcium glycerophosphate, is a revolutionary remineralizing agent that is well-known for its ability to provide calcium and phosphate ions that are bioavailable. Increasing the mineral saturation in the oral environment and providing support for the natural healing processes of teeth are two of the ways in which these ions are vital for boosting the remineralization of enamel. Studies conducted in clinical settings have demonstrated that CaGP has the ability to enhance the hardness of enamel and reduce sensitivity after dental operations [11].

Casein Phosphopeptide-Amorphous Calcium Fluoride Phosphate (CPP-ACFP): CPP-ACFP is a well-known drug that combines the benefits of fluoride with the potential benefits of bioavailable calcium and phosphate. Amorphous calcium phosphate and fluoride are both stabilized by the casein phosphopeptides that are included in this formulation. This makes it easier for these substances to be delivered to regions of demineralized enamel. It has been proven that CPP-ACFP is very efficient in reducing hypersensitivity and inhibiting the advancement of caries in hypomineralized teeth. This compound is widely found in dental creams and varnishes [12].

Placebo and Control Treatments: In clinical studies, the control groups are often given placebo treatments or normal fluoride toothpaste that does not include any extra remineralizing agents. This methodology is very necessary in order to assess the efficacy of active agents in relation to the practices that are considered to be standard for oral hygiene [13].

Objective

1. Examine the effects of remineralizing agents on enamel integrity, sensitivity, and caries development in hypomineralized teeth.
2. Assess the safety, acceptability, and compliance of remineralizing therapies in pediatric dentistry.

2. MATERIALS AND METHOD

This study follows all Declaration of Indian guidelines for human subject research. Over three months, laser fluorescence analysis was utilized to assess the remineralization of defined hypomineralized opacities in incisors affected by MIH. The study employed a randomized controlled trial. Participants were randomly assigned to intervention or control groups using gender-based randomization. The research method was consistently applied throughout the investigation. MIH patients were enrolled from the Department of Pediatric and Preventive Dentistry at Awadh Dental College. Parents or guardians were sought for informed consent after a detailed description of the study. From January to May 2024, this inquiry was centralized.

ANOVA for repeated measurements with between-group factors was used to prospectively calculate the sample size using G*Power version 3.1. CPP-ACFP reduced fluorescence loss by 0.9% (SD = 0.9%) during 12 weeks, mitigating MIH lesions. This research calculated power using this finding. A minimum sample size of 50 persons, uniformly distributed between two intervention groups and one control group, was calculated with a significance threshold of $p < 0.05$.

3. RESULT AND DISSECTION

Procedures for Intervention and Randomization

One pediatric dentist with a Bachelor of Dental Science degree was responsible for evaluating and enrolling all of the participants who were eligible and met the inclusion criteria. Prior to the commencement of the research project, a randomization list that was produced by a computer was compiled and safely kept beforehand. The participants were then randomly assigned to one of three groups, which were CaGP, CPP-ACFP, or control, in accordance with the randomization scheme that had been created. The same investigator was responsible for carrying out the assignment, and the information on the allocation was stored in a cabinet that was locked in order to guarantee that the allocation method was kept a secret. Each participant was given a one-of-a-kind identifying number as well as a sequential number that was determined by the sequence in which they visited the site. This ensured that their anonymity was maintained. The data analysis was carried out in a way that was blinded with regard to the allocation of groups.

Listed below are the intervention groups that were used:

1. **CaGP Group** – R.O.C.S.® Medical Minerals Gel, which is manufactured by R.O.C.S. and contains CaGP, magnesium, and xylitol, was delivered to the recipient. Trading GmbH in Moga, Punjab (n = 27).
2. **CPP-ACFP Group** – A shipment of GC MI Paste Plus™ was received from Fazilka, Punjab. This product contains fluoride (0.2% NaF) and 10% casein phosphopeptide-amorphous calcium phosphate. (n = 16).
3. **Control Group** – Participants maintained their regular oral hygiene regimen by utilizing a standard fluoridated toothpaste (Colgate, 1450 ppm F, Colgate Oral Pharmaceuticals, Malerkotla, Punjab) (n = 10).

Both verbal and written instructions were given to all of the participants and their guardians on the correct practices for dental hygiene as well as the precise way for utilizing the different items that were allocated for the research. Their oral hygiene routine consisted of brushing their teeth twice a day with fluoride toothpaste (Colgate Total, 1450 ppm F) for a minimum of two minutes, using either a manual or electric toothbrush. This was the instruction that they were given. Following the directions provided by the manufacturer, children were instructed to apply the appropriate remineralization product to the tooth surfaces using a finger that was clean and dry. This had to be done after they had finished cleaning their teeth. This application was to be performed twice daily, once in the morning and once before retiring to bed at night. The paste was to be left in the mouth during the night in order to assist slow breakdown.

It was recommended that parents supervise the application procedure and make certain that a minimum of a pea-sized quantity was applied to the teeth in both maxillary and mandibular arches. After the application, it was recommended that children refrain from rinsing, eating, or drinking for a minimum of thirty minutes. Parents were asked to bring the product that had been assigned to them to each follow-up meeting and to report on how they used the product. This was done in order to monitor compliance. During the course of the trial, the use of any extra fluoride products was strictly prohibited. It was taught to the study team that they should promptly tell them in the event that their children need any restorative dental procedures.

Measurements made with the Laser fluorescence

Laser fluorescence technology was utilized in order to conduct a quantitative evaluation of the remineralization process. A red diode laser with a wavelength of 655 nm was utilized in this technique. The laser was directed to the surface of the tooth by means of a fibre optic bundle and a specialized tip. The process of demineralization in enamel causes a drop in fluorescence, and the intensity of the laser beam that is reflected back into the enamel indicates the depth of the lesion; a greater reflection indicates a more thorough lesion. An increased protein content inside the enamel and/or the scattering effect of the laser induced by the non-homogeneous structure of the enamel have both been hypothesized to be possible explanations for the higher loss of fluorescence that occurs in hypomineralized enamel.

First, the teeth were separated, and then the DIAGNOdentTM Pen Indian was used with its Type A probe to collect quantitative data from the hypomineralized lesions. This was done in order to carry out the laser fluorescence measurements. A ceramic block was used to perform calibration on the device in accordance with the instructions provided by the manufacturer before each measurement session. Immediately following the completion of the calibration process, the probe was carefully positioned on the smooth enamel surface and then moved over the lesion until the highest fluorescence score was attained. This highest possible score was reported for each of the measurement sites. In order to ensure the accuracy and reliability of the readings, a training phase of two weeks was carried out, during which the device was utilised on a variety of different opacities. It was also possible to collect control measures on teeth that were not impacted as well as on healthy portions of the same teeth that were affected. In order to get the average score for each hypomineralized lesion, scores were gathered from three different locations: the mesial, the distal, and the central areas.

The results of the laser fluorescence measures were obtained at two different intervals in time: the first month, which served as the baseline, and three months after the remineralization therapy had been administered. The laser fluorescence threshold, which was set between 5 and 20, was utilised in order to arrive at an evaluation of the depth of the faults.

Statistical analysis was carried out using SPSS version 22, which was developed by SPSS Inc. in Chicago, Illinois, United States of America. Descriptive statistics were reported in the form of the mean plus or minus the standard deviation (SD." The Analysis of Variance (ANOVA) for repeated measurements was utilized in order to evaluate the efficacy of the remineralization agents for comparisons within the same group. In instances where it was determined that there were significant differences, the Student-Newman-Keuls multiple comparison test was utilised to identify particular differences among the means of the groups ($p < 0.05$).

A total of 401 hypomineralized teeth were included in the study, which was conducted on 102 children who were first examined for MIH. The participants comprised 28 girls (representing 53% of the total) and 25 boys (representing 47% of the total). The participants' ages ranged from 8 to 12 years, with a mean age of 9.34 ± 1.4 years. The clinically diagnosed levels of MIH were used to categorise these children. The lesions that were detected were categorized as white/creamy and yellow/brown defined opacities that exceeded 1 mm in diameter while remaining non-cavitated. All of the participants were randomly assigned to one of three groups: Group 1 (CaGP) consisted of 27 children with 197 teeth, Group 2 (CPP-ACFP) included 16 children with 125 teeth, and Group 3 was the control group, consisting of 10 children with 79 teeth who used F toothpaste. The process of remineralization was evaluated using Laser Fluorescence at the intervals that were specified.

Lesion Changes Assessed by Laser Fluorescence

There were no significant variations in laser fluorescence scores across the groups when the results of the initial evaluation were compared ($p > 0.05$). Despite this, the repeated-measures analysis of variance (ANOVA) revealed that there were notable differences in the scores of the laser fluorescence over time and among the different groups ($p < 0.001$).

In the course of the three-month period, people who had laser fluorescence scores that were either below 20 or over 20 indicated remineralization. This was determined by reviewing the scores at the baseline. Both experimental groups had a noteworthy improvement in laser fluorescence scores, as shown by a statistically significant increase ($p < 0.05$). In the control group, a similar pattern was found, where laser fluorescence values below 20 also suggested remineralization over time. Notably, substantial changes were documented ($p < 0.05$) in the laser fluorescence scores [**Table 1**].

Table 1: Comparisons of Laser Fluorescence Mean±SD across Study Period

Age Group	Group	Baseline Mean±SD	1st Month Mean±SD	3rd Month Mean±SD	p-value
≤20	CaGP	8.10 ± 5.01	7.53 ± 4.97	6.99 ± 5.03	0.0014
	CPP-ACFP	8.19 ± 5.72	7.07 ± 4.93	6.43 ± 4.42	0.027
	Control	6.13 ± 2.08	4.94 ± 3.15	5.37 ± 2.86	0.001
>20	CaGP	36.66 ± 19.42	30.30 ± 15.96	25.20 ± 14.08	0.003
	CPP-ACFP	38.89 ± 21.27	32.82 ± 21.95	32.56 ± 22.84	0.045
	Control	24.39 ± 12.81	24.08 ± 10.91	20.75 ± 10.62	0.221

The CPP-ACFP showed the most significant percentage change from the baseline to the follow-up measurements, with control and CaGP coming in second and third, respectively, in lesions that had scores that were lower than 20. On the other hand, when it came to lesions that had mean % changes in laser fluorescence scores that were greater than 20, those with CaGP had the biggest percentage changes, followed by those with CPP-ACFP and control, in that order [**Table 2**].

Table 2: Mean Laser Fluorescence Percentage Change Over Study Time

Age Group	Comparison	Mean Difference	p-value
≤20	CPP-ACFP – CaGP	4.92	0.088
	Control – CaGP	4.28	0.251
	CPP-ACFP – Control	0.63	0.855
>20	CaGP – CPP-ACFP	14.27	0.003
	CaGP – Control	25.69	0.002
	CPP-ACFP – Control	11.45	0.254

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: Mean percentage change in Laser Fluorescence scores compared across groups

Age Group	Group	Accumulated Mesurements	Average 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

For either of the two groups, there were no negative impacts or harms that were experienced by the participants, which had an impact on the participants' overall health.

4. DISCUSSION

The goal of preventive dentistry is to prevent the formation of first carious lesions, including as white spot lesions, developing enamel defects, and regions of hypomineralization, before they may proceed to cavitation. When it comes to managing incisors that have been affected by MIH, there are a number of therapeutic options accessible. The application of remineralization procedures that adhere to the principles of minimally invasive and preventative dentistry is the primary aim in the management of non-cavitated hypomineralized enamel. Although it is common knowledge that high fluoride concentrations can facilitate the remineralization of the upper layer of demineralized enamel, this effect does not extend to the deeper layers of the enamel [14]. As a result, it is recommended to make use of fluoride concentrations that are lower since these concentrations improve the uptake of calcium and fluoride ions from saliva, which in turn strengthens the process of deeper remineralization. Not only that, but there is a growing interest in the investigation of alternate remineralization agents in addition to fluoride [15].

Although improving the mineralization of MIH-affected teeth after eruption has been demonstrated in several in vivo and in vitro studies, complete resolution remains elusive due to the depth and/or thickness of the lesions [16]. Using CPP-ACP products for a long time can help remineralize teeth that have been impacted by MIH. This is particularly true in the early stages, when the enamel on freshly erupted teeth is still developing. After an eruption, the mechanical characteristics of MIH enamel may be improved according to several studies that found that CPP-ACP speed up the maturation process. Surface porosity reduction has other potential benefits, including reduced susceptibility to heat and tactile stimulation and cavities. CPP did not change the in-vivo surface morphology of molars impacted by MIH [17]. Their findings suggested that MIH teeth, which lack these minerals, might benefit from a mix of phosphate and calcium ions supplied by bigger molecules. Although it may take some time for the effects of CPP-ACP to become apparent, it does help MIH enamel mature. Overall, the results of this study cast doubt on the original hypothesis and call for additional research into fluoridated CPP-ACP products and adjustments to application protocols, as MIH molars showed improvement after applying Ca-PO4 casein. Aesthetic outcomes for untreated incisors and functional restorative therapies for MIH molars may be improved with procedures utilising CPP-ACP, even if there are no in-vivo investigations or supplementation trials available at this time. However, there is a lack of data on the long-term efficacy and best practices for using CPP-ACP to treat MIH lesions [18].

Following four treatments of fluoride varnish, Restrepo et al. discovered that the remineralization of MIH lesions in anterior teeth did not experience any effects that were statistically significant [19]. On the other hand, the results of our study demonstrated that the utilization of fluoride toothpaste had a positive influence on the remineralization of incisors that were impacted by MIH. Even though it has been proven that CPP-ACFP is successful in preventing and correcting early lesions in vitro, there is still a lack of adequate convincing information about its efficiency in treating MIH lesions in vivo. Furthermore, the long-term consequences of this remineralizing agent are still little understood. It is possible that the fluoride ions present in CPP-ACFP prevented calcium and phosphate ions from penetrating the lesions to a greater depth. As a consequence, there was an initial precipitation of these ions on the enamel surfaces that were at the surface of the tooth [20]. The data that we obtained are consistent with this observation, since we found that the CPP-ACFP group had higher amounts of remineralization, particularly for laser fluorescence scores that were lower than 20. According to the findings of a study that was carried out by Bakkal and colleagues, both CPP-ACP and CPP-ACFP were able to dramatically reduce laser fluorescence scores within one month of their treatment [21].

According to the findings of this pilot investigation, CPP-ACP and CPP-ACFP were able to successfully contribute to the decrease of hypomineralization on enamel surfaces that were impacted by MIH over the course of one month. In a similar vein, the results of our research suggested that CPP-ACFP has the potential to be an efficient remineralization agent for incisors that have been affected by MIH during a therapy period that is quite short-term. In addition, CaGP, which is an organic polyphosphate, possesses the ability to cling to enamel surfaces by means of the phosphate radicals that are present in its composition. Throughout the demineralization process, CaGP performs its duties at a variety of phases. The majority of the phosphate groups are neutralized by calcium and hydroxyl groups from hydroxyapatite molecules when the organic phosphate connects to the enamel surface. This results in the release of calcium ions from CaGP in the vicinity of the enamel surfaces [22].

According to the findings of studies that investigate the interactions between calcium CaGP and sodium mono fluorophosphate, the proportion of mono fluorophosphate to CaGP is an essential factor in the demineralization of hydroxyapatite and enamel [23]. The utilization of sodium mon fluorophosphate and CaGP in isolation is commonly acknowledged to be much less successful than the utilization of both of these substances in conjunction with one another. Especially noteworthy is the fact that CaGP has a powerful protective impact on enamel, regardless of the presence of fluoride [24]. Grenby and Bull conducted a research that demonstrated that pre-treating hydroxyapatite discs with sodium fluoride resulted in a sustained protective effect against demineralization. This was discovered through the findings of the study. According to the findings of their research, even though fluoride and CaGP function through separate processes, the effects of both of these substances might be complimentary to one another and cumulative [25]. There are a number of potential pathways that contribute to the cariostatic characteristics of CaGP, and it has been suggested that CaGP interacts directly with the outer layers of enamel. Direct contact with enamel, buffering the pH of plaque, lowering plaque bulk, changing plaque metabolism, and elevating calcium and phosphate levels inside the plaque are some of the processes that are involved in this process. The anti-caries properties of CaGP have been demonstrated in vivo on a consistent basis, and it has the potential to offer protective advantages when given on a regular basis and at larger concentrations. Additionally, fluoride and CaGP appear to have an additive impact, which most likely manifests itself in the form of unique processes that influence enamel breakdown. It is clear and noteworthy that the possible anti-caries benefits, particularly those resulting from buffering plaque acids, are being considered [26].

Results for in-situ demineralization were similar between the positive control (1,100 ppm fluoride toothpaste) and the low-fluoride toothpaste containing 0.25% CaGP. Clinical results from using low-fluoride toothpaste boosted with CaGP are comparable to those from using a formulation with 1,100 ppm fluoride, according to the research [27]. Zaze et al. shown that in-vitro enamel demineralization could be prevented just as well with a low-fluoride toothpaste (500 μg fluoride/g) containing 0.25% CaGP as with a 1,100 μg fluoride/g toothpaste [28]. While Rezende et al. discovered no statistically significant differences between the groups in their assessment of enamel surface remineralization using different fluoride solutions in conjunction with CaGP (0.13%), they did find that CaGP significantly improved remineralization when compared to the control group [29]. Our research also found that when employing CaGP, hypomineralized portions of incisors impacted by MIH remineralized, which aligns with our results. These specific components have never been the subject of an examination prior to this one.

Compared to the CPP-ACFP and control groups, CaGP resulted in considerably higher remineralization in teeth with laser fluorescence scores surpassing 20 after three months ($p<0.01$). This is probably because CaGP has a high attraction to the hydroxyapatite structure, which lets it pass through the fluoride-induced surface remineralization layer and have its impact. Clinical data demonstrating the anti-caries actions of PO4 salts in slowing the advancement of carious lesions is still missing, despite the favourable outcomes linked with toothpastes containing CaGP [30].

Hypomineralized teeth with high laser fluorescence scores may have less mineral in MIH-enamel due to their organic nature. The changed prismatic structure and intrinsic inhomogeneity of hypomineralized enamel may explain these higher laser fluorescence readings [31]. MIH-enamel's darker pigmentation may also increase laser fluorescence scores. This study found that MIH lesions with higher baseline laser fluorescence scores had less mineral content. High laser fluorescence scores in non-carious teeth indicate hypomineralized enamel, either due to protein content or light scattering due to its uneven shape. Bacterial metabolites may affect fluorescence, especially in hypomineralized enamel, although a higher laser fluorescence score indicates damaged enamel. Higher laser fluorescence scores imply impaired enamel integrity regardless of fluorophore. Beyond 20 laser fluorescence scores, organic material may not alter hypomineralized enamel's mechanical qualities [32].

5. CONCLUSION

Utilizing laser fluorescence measures, researchers found that remineralization drugs such CPP-ACFP and CaGP helped improve MIH lesions in children after three months of therapy. Our findings show that CPP-ACFP and CaGP based therapies successfully helped incisors affected by MIH remineralize their hypomineralized enamel. The findings of this study provide more evidence that agents containing minerals can benefit teeth impacted by MIH by shedding light on the process of enamel remineralization. Additional remineralising agents, longer treatment durations, and bigger sample sizes should be considered for future study to strengthen the validity of these findings. These medications offer a non-invasive and patient-friendly approach to treating early carious lesions in clinical practice. The higher efficacy of CaGP and CPP-ACFP must be confirmed by additional in-vivo research. Additionally, the reliability of laser fluorescence measures in assessing MIH lesions and tracking the effects of remineralising agents must be established.

Conflicts of interest: Nil

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