Effect of Fluoride Varnish on Enamel Remineralization in Anterior Teeth with Molar Incisor Hypomineralization

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Objective: *The objective of this study was to investigate the effect of fluoride varnish on remineralization of anterior teeth affected by Molar-Incisor Hypomineralization (MIH) by means of Quantitative Light-Induced Fluorescence- QLF.* ***Study design:*** *Fifty-one healthy 9 – 12- year-old children were selected according to different clinically diagnosed levels of MIH, proposed by the European Academy of Pediatric Dentistry (2003) (considering the most severe lesion per patient, n= 51 lesions), and randomly divided into two groups: (1) four applications of 5% NaF varnish, with one-week interval, and (2) usual home care- control. At each visit, the mean change in fluorescence and area of lesion were measured by QLF. The data were analyzed by repeated measures ANOVA and Tukey's test.* ***Results:*** *All patients showed enamel alterations in first permanent molars and incisors, frequently with two molars affected by MIH (41.1%). There was no statically significant difference in the mean of fluorescence and area of lesion between groups over the studied time.* ***Conclusion:*** *We observed no favorable effect on the remineralization of MIH lesions in anterior teeth after four applications of fluoride varnish.*

Key words: *Fluorescence; Fluoride; Molar Incisor Hypomineralization; Tooth remineralization.*

INTRODUCTION

Molar Incisor Hypomineralization (MIH) is an enamel defect that mainly affects permanent first molars, while permanent incisors are often affected to a lower degree and with variable severity. The etiology is unclear, however, etiological associations with systemic conditions, environmental insults during the child's first 3 years of life, and genetic variations have been implicated.[1-3]

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Clinically, MIH may present as discrete opacities, with color ranging from white to yellow-brown, asymmetrical in appearance and sharp demarcation between sound and affected enamel.[4] Commonly the enamel of one molar can be severely affected whilst the enamel of the contra-lateral molar is clinically unaffected, or has only minor defects.[5] These teeth are porous,[6] impacting on oral health, which can lead to unusual cavitation, enamel disintegration, hypersensitivity, secondary caries, atypical restorative treatments, loss of fillings and extraction of the affected teeth. Consequently, the affected teeth often require repeated treatment.[7]

Treatment of teeth affected by MIH consists of a minimally invasive approach by reinforcing and protecting the existing dental structure.[8-10] Caries remineralizing agents are often recommended for MIH management in order to increase mineral content of the hypomineralized areas, however, scientific evidence of the effectiveness of this treatment is still limited. After over 50 years of clinical success, fluoride serves as the gold standard remineralizing agent. When fluoride is applied on teeth, there is precipitation of minerals (calcium fluoride-like deposits and fluorapatite).[11] Calcium fluoride serves as a source of fluoride for the formation of fluorapatite, thereby inhibiting demineralization and enhancing remineralization.[11]

Researchers have shown an increasing interest in non-destructive methods for the quantitative assessment and longitudinal monitoring of mineral changes in enamel, such as Quantitative Light-Induced Fluorescence- QLF. QLF is a system based on the measurement of loss of fluorescence subsequent to enamel demineralization. QLF has also shown the ability to detect and quantify

changes in mineral content and size of lesions by demonstrating a dose response between fluoride and non-fluoride products in both in vitro and in vivo studies.[12-14]

Due to the lack of evidence of the effectiveness of treatments for remineralizing teeth affected by MIH, the aim of the present study was to investigate the effect of fluoride varnish on remineralization of MIH lesions, by means of QLF.

MATERIALS AND METHOD

The present study was approved by the Research Ethics Committee of the Araraquara School of Dentistry, Univ. Estadual Paulista- Unesp under the Protocol No.11/08, and was conducted in accordance with the principles of medical research involving human subjects described by the Declaration of Helsinki (as revised in Tokyo 2004). The parents were informed about the purpose of the study and gave written consent for their children to participate.

A total of 51 patients (n = 51 lesions) of the Orthodontics and Pediatric Department of the Araraquara Dental School - Unesp (35 males and 17 females, 9 – 12 years old, mean age = 10.25 ± 1.14) were selected according to different clinically diagnosed levels of MIH. The power analysis revealed that 25 lesions would be needed in each group to detect a 20 % difference between an intervention and a control group with α- and β-values set at 0.05 and 0.2, respectively.

For the diagnosis of MIH, the judgment criteria proposed by the European Academy of Paediatric Dentistry (EAPD) was used. Child-level of MIH was defined as present whenever any of the first permanent molars exhibited demarcated enamel opacity. Regarding the severity of the lesions, teeth presenting demarcated opacities were considered to have mild MIH; moderate MIH included lesions in teeth with rough and broken enamel. Severe defects included the presence of hypomineralized lesions associated with loss of dental structure, atypical restorations and teeth extracted due to MIH.[15] All examinations were conducted by two experienced calibrated examiners (Kappa value = 0.93).[1]

One lesion per patient was selected, based on the most severe opacity among anterior teeth affected by MIH.[15, 16] Only demarcated opacities larger than 2.0 mm in diameter were considered. Enamel opacities were also recorded according to color shades of white, yellow and brown.[16] The selection criteria for anterior teeth were: fully erupted, with demarcated opacity, no previous treatment of the lesions, without caries lesion and patients living in Araraquara, São Paulo, Brazil. Patients undergoing orthodontic treatment with fixed appliances, with loss of tooth structure, fillings and/ or with dental fluorosis were excluded.

Based on a randomization list, the participants were randomly divided into 2 groups: (1) four applications of 5 % NaF varnish with one-week interval (n= 26 lesions) and (2) usual home care- control (25 lesions).

The teeth were first cleaned using brushing and pumice slurry. Thereafter, the teeth were dried and treated with 5% NaF varnish (Duraphat®, Colgate Palmolive, Hamburg, Germany). The patients were instructed not to eat anything, or to brush their teeth for at least four hours after the treatment.

In the control group, no professional treatment was performed. The teeth were only rubbed with cotton swabs imbibed with deionized water.

All patients received instructions with respect to oral hygiene and diet. During the study, fluoride toothpaste (Colgate Total with 1.450 ppm fluoride) was used for oral hygiene twice a day. Study participants were also instructed not to use any other fluoride or/and antiplaque agents.

QLF images were taken at the beginning of the study (baseline) and at each weekly visit (4 weeks). The labial surfaces of the permanent incisors were illuminated by diffuse blue-green light (λ= 488 nm, 10 ± 20 mW cm^{-2}) from an argon ion laser, conducted by an optical fiber to the measuring area. A micro-CCD-video camera (Panasonic WV ± KS 152, length 50 mm, diameter 17 mm) equipped with an orange high-pass filter to exclude scattered light, was used to produce images of the hypomineralized lesions. The images were captured in a dark room. Teeth were dried for 15 seconds with air from a triplex syringe prior to image capture. Video repositioning software was used to ensure that images were automatically captured when the correlation was higher than 0.95, to ensure consistent capture areas. The images were analyzed using a commercial software program (Inspektor QLF 1.97, Inspektor Research Systems, Amsterdam, The Netherlands) to determine the change in fluorescence (ΔF, %) and extension of the lesion (area; mm^2).

The statistical analyses were performed using the Statistical Package for Social Sciences 17.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The normal distribution of data was checked using the Shapiro-Wilk test. The data comprised two variables, which were repeated five times. The treatments were compared using repeated-measures ANOVA and Tukey's test, considering a 5% significance level.

RESULTS

No adverse events were reported during the study. All patients enrolled in this study showed enamel alterations in first permanent molars and permanent incisors, frequently with two molars affected by MIH (41.1 %).

Of the total number of teeth affected by MIH (n= 158), 106 (67 %) were in the maxilla and 52 (33 %) in the mandible. The teeth most commonly affected were the maxillary permanent first molars, followed by the mandibular first molars, and maxillary and mandibular central incisors. Among the molars the maxillary left permanent first molar was the tooth most affected by MIH, and among the incisors, the maxillary right permanent central incisor was the tooth most affected (Table 1).

When considering the severity among children, the following was observed: 38 patients (74.5 %) had demarcated opacities (whose color ranged from white to brown, with yellow being the most common), 4 patients (7.9 %) had enamel breakdown, 9 patients (17.6 %) exhibited atypical restorations and there were no cases of tooth extraction.

Of the 51 teeth, 27 (53 %) were maxillary central incisors, 15 (29.4 %) maxillary lateral incisors and 9 (17.6 %) mandibular central incisors. The yellow MIH opacities were the most frequent (62 %), followed by white (23.5 %) and brown (14.5 %).

The results of the Quantitative Light-Induced Fluorescence measurements are summarized in Table 2 and Table 3. There was no statistically significant difference between groups at baseline in the mean of fluorescence (ΔF, %, p=0.08) and extension of MIH lesions (ΔQ, % x mm^2, p=0.11). In the fluoride varnish group and the control group there were no statistically significant changes over time in both average change in fluorescence and extension of MIH lesions.

Table 1. Distribution of teeth affected by MIH, according to arch and hemiarch affected.

Tooth affected	Maxillary Arch			Mandibular Arch		
	Right hemiarch	Left hemiarch	Total	Right hemiarch	Left hemiarch	Total
Central incisor	18	9	27	2	7	9
Lateral incisor	5	10	15	-	-	-
First molar	28	36	64	18	25	43
TOTAL	51	55	106	20	32	52

Table 2. Mean and standard deviation of fluorescence over studied time (ΔF, %).

Group	Baseline	2nd visit	3rd visit	4th visit	5th visit
Fluoride varnish	-7.47 ± 0.43 aA	-6.84 ± 1.23 aA	-7.52 ± 0.92 aA	-6.52 ± 1.14 aA	-6.32 ± 0.50 aA
Control	-7.22 ± 0.40 aA	-7.54 ± 0.87 aA	-7.31 ± 0.95 aA	-6.76 ± 1.17 aA	-6.43 ± 0.64 aA

Different lower case letters within the same column show significant differences among the treatments. Different capital letters within the same row show significant differences among the periods of remineralization (ANOVA, $p<0.05$).

Table 3. Mean and standard deviation of extension of MIH lesions over studied time (ΔQ, % x mm²)

Group	Baseline	2nd visit	3rd visit	4th visit	5th visit
Fluoride Varnish	-0.87 ± 0.35 aA	-0.83 ± 0.38 aA	-0.95 ± 0.49 aA	-1.08 ± 0.23 aA	-0.89 ± 0.34 aA
Control	-0.92 ± 0.25 aA	-0.88 ± 0.32 aA	-1.03 ± 0.27 aA	-0.98 ± 30 aA	-0.87 ± 0.29 aA

Different lower case letters within the same column show significant differences among the treatments. Different capital letters within the same row show significant differences among the periods of remineralization (ANOVA, $p<0.05$).

DISCUSSION

Recently, the challenge to dentists has been the diagnosis and treatment of MIH. Differential diagnosis is mandatory, in order not to confound it with other enamel defects. MIH must be differentiated from dental fluorosis, which is characterized by diffuse opacities that affect the teeth symmetrically,[17] and amelogenesis imperfecta that differs in the distribution of the opacities. Whereas in MIH, molars are rarely affected to the same degree of intensity; in amelogenesis imperfecta the entire dentition is affected and there is genetic involvement.[18]

Considering the characteristics of the patient with MIH, the effective management teeth affected by MIH is an ongoing issue for the majority of clinicians and researchers. Published guidelines and recommendations for treatment include the application of remineralizing agents, despite the lack of evidence that these lesions have the capacity to increase their mineral content.[8] Fluoride varnish (Duraphat) was chosen as the remineralization agent, as previous studies have shown that it releases a high number of calcium and fluoride ions, has the capacity to create a reservoir of fluoride ions that are slowly released,[19] is safe, and is used for the control of hypersensitivity, frequently associated with MIH.[20]

Transverse microradiography is considered the gold standard for quantifying changes in mineral content in enamel. However, for this analysis the technique requires preparation of thin tooth sections, and therefore, cannot be used to monitor the remineralization of teeth with MIH. In this in vivo study, the QLF method was used to monitor changes in fluorescence and extension of lesions in anterior teeth treated with fluoride varnish. The high sensitivity of the method has been confirmed in several studies.[21] QLF has been validated against TMR for quantifying demineralization and remineralized areas, with the use of a commercial QLF system showing moderate to high correlation.[21, 22] Recently, it was demonstrated that QLF is capable of distinguishing treatments with different levels of fluoride.[14]

The application of fluoride varnish on MIH lesions showed no significant changes in both mean levels of fluorescence and area of lesion over time. One reason may be due to the architectural organization and protein/mineral content of the affected enamel, which makes it difficult for any attempt of mineral incorporation to succeed/ occur.[23] It should also be considered that the QLF method quantifies the mineral loss and size of sub-surface enamel lesions, and MIH is an enamel defect that may involve a large enamel thickness. However, alterations in fluorescence can be detected in MIH lesions due to the increase in enamel porosity. The light scattering in the lesion, which is much stronger than it is in sound enamel, causes the light path in the lesion to be much shorter than in it is sound enamel, thus the light absorption per volume is smaller in the lesion, and therefore the fluorescence is weaker.[24]

On the other hand, it should be considered that *enamel defect* is not synonymous with intensive therapy with remineralizing agents, whereas it is synonymous with prevention, control and periodic assessment. Adequate oral hygiene supplemented with fluoride toothpaste may be sufficient for the management of MIH opacities. The result of this study does not suggest that fluoride varnish is not recommended in the treatment of MIH lesions. Due to the association between dental caries and MIH,[18] professional fluoride applications are an effective complementary method to reduce dental caries by reducing the solubility and enhancing remineralization of dental enamel by the incorporation of available fluoride into the tooth structure during acid attacks.[25] Moreover, the use of fluoride varnish is important to prevent loss of structure and control the hypersensitivity, at present considered to be associated with the condition.

Recently, the use of a commercial product containing Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) was proposed. The molecule is derived from casein, part of the protein found in cow's milk. Its activity is due to a part of the casein protein called casein phosphopeptide (CPP), which carries and stabilizes calcium and phosphate ions in the form amorphous calcium phosphate (ACP).[26] Some authors suggest that CPP-ACP may accelerate and increase the potential maturation of MIH enamel structure, improving its proprieties after tooth eruption. Decreasing the surface porosity may improve the sensitivity by decreasing thermal/tactile stimulation and reducing caries risk.[9, 10] However, there is a lack of evidence as regards the long-term effectiveness and protocols of products containing CPP-ACP in MIH lesions.

Future investigations may increase the experimental time to

determine the effectiveness of remineralizing agents over time. Products such fluoride varnish, fluoride containing polyvalent metal, titanium tetrafluoride (TiF_4) and products containing CPP-ACP could be tested and compared on teeth affected by MIH. Research on these products could justify their use to improve the mineral content of the enamel and prevent the development of caries lesions and loss of tooth structure, especially in molars, as well suggest clinical protocols to manage MIH without surgical intervention and avoid the high biological, social, psychological and financial cost associated with the condition.[10]

CONCLUSION

We observed no favorable effect on the remineralization of MIH lesions in anterior teeth after four applications of fluoride varnish.

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