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Assesment of Remineralization of Hypomineralized Enamel Lesions Using Self-Assembling Peptide Using Laser Fluorescence- A Pilot Study

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Abstract

The hypomineralized enamel lesions have 20% less mineral content, substantially reduced calcium and phosphorus ratio, and an increased carbon content which increases the likelihood of demineralization. The application of remineralizing agents on these lesions have been tried. In the present pilot study, to assess the change in mean fluorescence after the application of self-assembling peptide (SAP) on hypomineralized enamel lesions on ten MIH-affected incisors using laser fluorescence. After a single application of SAP, there was a decrease in the mean fluorescence score record with Diagnodent at two-time intervals i.e., baseline and one-month post-application. Thus, it can be concluded that the SAP application could use as a viable treatment option.

Keywords: Molar Incisor Hypomineralization, CPP-ACP, Self-assembling peptides, remineralization, Laser fluorescence.

INTRODUCTION

The name 'Molar Incisor Hypomineralisation' (MIH) was suggested by Weerheijm at the congress of the European Academy of Pediatric Dentistry, 2001 which is defined as enamel hypomineralisation of systemic origin of 1-4 permanent first molars, frequently associated with affected incisors (Weerheijm KL. 2003). Clinically, these hypomineralised enamel defects present with altered translucency in localized demarcated areas of enamel (Kotsanos et al.2005) on a continuous spectrum ranging from demarcated creamy white or yellow opacities to brownish defects with or without loss of enamel (Weerheijm *et al*. 2001). It is a common problem and one in 10 children could be affected by MIH.

Ultrastructurally, enamel rods in MIH affected teeth are widely and irregularly placed, which makes it porous, and the comparative less mineral content makes it more susceptible to breakdown due to reduced mechanical properties (Jälevik B, Norén JG. 2000). Tooth remineralization is a physiological process in which calcium and phosphate ions are sourced to promote their deposition into crystal voids of the demineralized enamel. Remineralizing the milder forms of hypomineralized enamel in MIH-affected teeth would be a viable treatment option for enhancing the mineral content and improving the mechanical properties of enamel.

The efficacy of available remineralizing agents has been evaluated on white spot lesions. However, there is a lack of data on their effect on MIH. A newer remineralizing agent Self Assembling Peptide (SAP) has shown remineralizing potential by the formation of the hydroxyapatite crystals denovo besides increasing the net mineral gain and inhibiting mineral loss (Cochrane *et al*. 2010). This agent undergoes well-characterized three-dimensional fibrillar scaffolds in response to specific environmental triggers (Gagnaire *et al*. 1996). The scaffold mimics proteins found in enamel during tooth development and provides a base to support hydroxyapatite crystallization around it to regenerate tooth enamel. It is a low viscosity isotropic liquid which switches to an elastomeric nematic gel at a pH of less than 7.4 (Aggeli *et al*. 1997). Its efficacy has been tested on early enamel carious lesions and is very effective. However, no study to the best of our knowledge has been conducted to date to see its efficacy in the remineralization of enamel hypomineralized lesions seen in MIH affected teeth.

The use of laser fluorescence in the form of Diagnodent has come up as a quantifiable measure of the hypomineralization in MIH-affected teeth. Higher laser fluorescence scores are positively correlated with, the increasing grades of MIH-associated surface opacities (highest for brown opacities and lowest for creamy white opacities). So, the present pilot study was the plan to assess the change in mean fluorescence after the application of SAP on hypomineralized enamel lesions on MIH-affected incisors using laser fluorescence.

MATERIAL AND METHOD

A present pilot study was approved by the institutional ethics committee of our institute. The study will be conducted on 10 hypomineralized enamel lesions on MIH-affected incisors in 8 to 16 years old children. Hypomineralized enamel defect of MIH with intact yellow, and brown localized demarcated opacities without any post-eruptive breakdown (PEB) will be included. Diagnosis of MIH will be made according to the EAPD 2003 criteria and the severity of the lesion will be calculated according to the Oliver 2014 criteria. The defect characteristics will be described, and the size of the lesion will be defined. The sample will be selected from the outpatient unit of pediatric dentistry of our institute. The patient and their parents were informed about the study objectives, written informed consent was obtained from the parents. Prior to the application of test agents, Oral prophylaxis was given to all the enrolled patients, the hypomineralized enamel lesions on MIH affected incisors were cleaned with prophylaxis paste and then deproteinization with 5% sodium hypochlorite solution and then etched with 37% orthophosphoric acid for 30 seconds to open up pores to the subsurface lesion, washed and dried. SAP was then applied on the lesions for 2 minutes ensuring that no portion of the agent was visible to the naked eye. Children were advised not to brush on the lesions for 4 days from the day of application to ensure that the 3-dimensional fibrillar scaffold was formed on the enamel and was not disturbed. During this period of 4 days, no mechanical cleaning was advised on the hypomineralized enamel lesions, and they were advised to do 0.12% chlorhexidine mouth wash for 4 days following which they were advised to brush twice daily with a fluoridated dentifrice with 1000 ppm F. The DIAGNOdent will be used to assess the remineralization of hypomineralized enamel of MIH affected incisors at two-time intervals i.e., at T0 (baseline) and T1 (1 month). After air-drying for about 5 seconds, three readings will be taken from each hypomineralised area and the average will be taken as the reading for each tooth at every time point aforementioned.

STATISTICAL ANALYSIS

Descriptive and inferential statistical analysis was done in the present study by using SPSS version 18 (IBM Corporation, SPSS Inc., and Chicago, IL, USA). Results on the continuous measurement will be presented on Mean ± SD. A p-value of less than 0.05 will be considered to be significant, two-tailed.

RESULT

The mean fluorescence score was recorded using Diagnodent on 10 hypomineralized enamel lesions of MIH-affected incisors. At baseline, the mean fluorescence was found to be 23.16± 4.56 and after a single application of self-assembling peptide, the mean fluorescence score was found to be 14.83±2.46. The decrease in the mean fluorescence score has shown the effective remineralizing potential of SAP on hypomineralized enamel lesions (Fig 1).

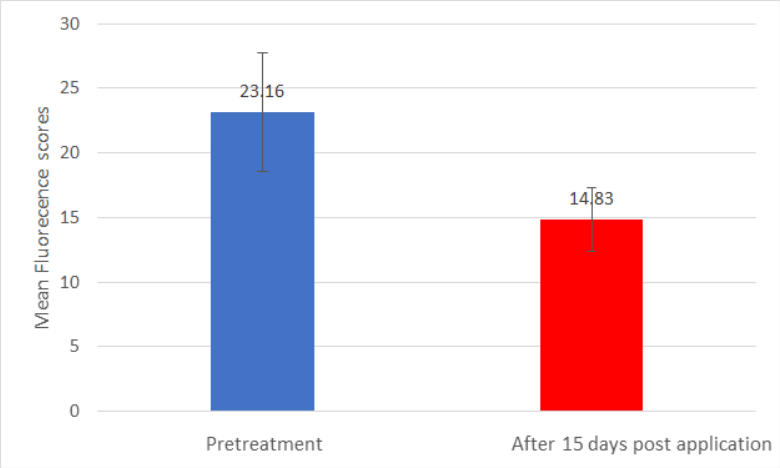

Fig-1: Comparison of mean fluorescence scores at baseline and after 15 days of post application

DISCUSSION

Self-assembling peptide consists of monomer with short sequences of amino acids that assemble and showed a distinctive physicochemical and biochemical activities which depend on their morphological features. The interaction of these short chains of the repeatable amino acid sequence is organized into a different

conformation and assembled into nanostructures like nanofibers, nanotubes, and nanovesicles.

In MIH, the hypomineralization is due to the non-degradation of the organic enamel matrix in the final stage of odontogenesis and thus the organic matrix occupies the space into which the mineral crystals should grow. Any form of treatment must first remove the organic matrix trapped within the MIH teeth, to gain space for mineral deposition. That is chemically possible with the use of 5% sodium hypochlorite which is classically known as deproteinization. A similar procedure has been used in the present pilot study to assess its effectiveness.

Manuel Restrepo *et al*. (2016) have also shown changes in mineral composition of hypomineralized enamel of MIH affected molars and incisors after application of fluoride varnish every 3 months using QLF. Berkant Sezer *et al*. (2018) have shown a significant change in Diagnodent pen scores from baseline to 3 months after regular application of CPP-ACPF on hypomineralized enamel of MIH affected anterior teeth. Studies done by J. Kirkham *et al*. (2007), Joshua D. Silvertown *et al*. (2016), M. Alkilzy *et al*. (2018), Sindhura V *et al*. (2018) showed that SAP is effective in remineralizing early enamel carious lesions by increasing the mineral content and Prashant Babaji *et al*. (2019) showed that it is better than CPP-ACP in remineralization.

The findings of the present study have also shown a significant change in mean fluorescence scores after a single application of self-assembling peptides using Diagnodent. An increase in scores indirectly correlated with the increased mineral content of these hypomineralized enamel lesions. Throughout this period, there were no adverse events have reported.

The main study that will follow this pilot will also consider the categories of potential patients classified according to EAPD 2003 and Oliver hypomineralized severity calculating index 2014.

Limitations

SAP is quite expensive material, which is not available in India, So, a smaller sample was selected for the pilot study to check whether it can be tried on hypomineralized enamel lesions as well. Thus, a smaller sample size and limited follow-up questioned its generalizability.

Future Prospects

- A long-term two or three parallel arm/s prospective clinical trial on a larger sample size, to assess and compare the remineralizing potential of the self-assembling peptide on the hypomineralized enamel lesions of MIH affected teeth with other remineralizing agents.
- The elemental change in their mineral content along with their ultrastructural features post-application of the self-assembling peptide.
- The effect of SAP can be compared after their application of white spot lesions and hypomineralized enamel lesions.

CONCLUSIONS

Within the limitations of the present pilot/ feasibility study, the following conclusions can be drawn:

- SAP could be an alternative viable preventive treatment option for remineralizing the hypomineralized enamel lesions.
- The study is feasible with changes to the protocol on a larger sample where it can be compared with other remineralizing agents.
- Laser fluorescence is a good instrument for monitoring short-term changes in mineralization stage.

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