Use of Ionized Monocalcium Phosphate and Enameline Derivatives to Reduce Dentin Hypersensitivity After Periodontal Therapy

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ABSTRACT: This study aimed to evaluate the effectiveness of using an ionized monocalcium phosphate and enamelin derivatives (IMP+ED) based mouthwash for the treatment of dentin hypersensitivity (DH) after scaling and root planing (SRP). 47 patients who reported DH after SRP treatment were included in this prospective cohort study. The Schiff Cold Air Sensitivity Scale (SCASS) was applied to classify their degree of pain in mild, moderate or intense at two times: after SRP (T0), and after one month of using the IMP+ED-based mouthwash (T1). The McNemar-Bowker test was used to compare the correlated proportions between both times (p<0.05). After the SRP therapy (T0), all the sample members reported pain distributed in the following manner: 12.8% were mild, 27.6% moderate, and 59.6% intense. At one month since treatment and with the use of the IMP+ED-based mouthwash (T1), the distribution of pain levels changed to 83% mild, 12.8% moderate, and 4.3% intense, this change was statistically significant (p<0.001). IMP+ED-based mouthwash produces a positive effect in reducing painful responses caused by exposure of the dentin tubules to the oral environment after SRP therapy.
INTRODUCTION

Dentin hypersensitivity (DH) is defined as a sharp and short pain that is caused by the exposure of the dentin to the oral environment. Such pain can arise due to thermal, chemical, osmotic, or tactile changes or stimuli, and cannot be classified as any other dental pathology (1). The presence of hypersensitivity is related to the caliber of the exposed tubules, which decrease depending on the age of the individual (2). Some of the risk factors associated with exposure of the dentin tubules include the loss of tooth enamel (due to direct trauma such as blows or abrasive tooth brushing) and the appearance of gingival recessions and concomitant root denudation (3).

DH seriously affects patient quality of life, since it leads to a continuous episode of pain that forces the patient to modify their eating and personal habits (4). Previous studies have reported a wide range of values for the prevalence of DH after scaling and root planing (SRP), ranging between 3% and 98% (5). Rane et al. (2013), reported that a 30% prevalence of DH increased to 67%-76% 1 week after periodontal therapy with SRP (6).

DH is conventionally treated with toothpastes, mouthwashes, and varnishes as adjuvants of pain control. These vary widely in terms of the active component used, examples of which include potassium nitrates and citrates, arginines, sodium fluorides, and calcium phosphates (7,8).

There is extensive literature on the efficacy of toothpastes and varnishes as part of the treatment against DH, but there is scant information specific to the use of mouthwashes as desensitizing agents (9-11).

The “Enamelin®” mouthwash (Operating System Select SA de CV) contains ionized monocal-
cium phosphate [Ca(H2PO4)2] and derivatives of the protein enamelin as active agents that act to lower DH. These components combine buffering properties and the ability of monocalcium phosphates to occlude exposed dentin tubules with the ability of enamelins to control (and initiate) the mineralization of the extracellular matrix (remineralization of dentin) and regulate growth, orientation, and the size of hydroxyapatite crystals in the enamel, thus achieving a powerful desensitizing effect (12,13).

This study aimed to evaluate the effectiveness of using a mouthwash with ionized monocalcium phosphate and derivatives of the protein enamelin (IMP+ED) for the treatment of DH after SRP in patients with periodontal disease.

STUDY POPULATION AND METHODOLOGY

This prospective cohort study was conducted with a non-random sample selection method. The cohort comprised patients with periodontitis who received periodontal therapy in a postgraduate clinic specializing in periodontics. The sample consisted of patients over 18 years of age admitted to the periodontics clinic to begin initial phase periodontal therapy. All subjects were systemically healthy or compensated, with asymptomatic gingival recessions, without DH but diagnosed with DH in at least one tooth after SRP treatment. The study excluded patients who were pregnant, those who had received any type of DH treatment in the 6 months prior to the intervention (SRP), those who had received dental whitening treatment during the 6 months prior to the intervention; those with teeth suffering from occlusion trauma, those with carious lesions with dentin involvement or fractured restorations, individuals with a history of gastric esophageal reflux or bulimia, those with a history of excessive intake of acidic foods, smokers, and patients who were under chronic treatment with any analgesic drug. Individuals who voluntarily decided to abandon the study were eliminated, as were patients who did not adhere to the indicated use of the mouthwash. Patients who during the period of use of the mouthwash had used a different type of desensitizing agent were also eliminated from the study. The study was conducted after obtaining approval from the Institutional Research Committee (SISTPROY FODO-2017-0007); the research was performed in adherence to the ethical guidelines and recommendations laid out by the Declaration of Helsinki, Finland (1964) and its modification by the World Medical Assembly in Korea (2008). All patients who met the inclusion criteria signed a voluntary and informed consent for participation.

The included patients underwent clinical management as follows: all patients underwent the conventional periodontal diagnosis protocol and were evaluated upon arrival at the periodontics clinic with a “North Carolina” periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA). Probing depth, bleeding on probing, amount of bacterial plaque, dental mobility, and furcation were recorded in the periodontogram model proposed by the University of Bern (periodontal chart-online, www.periodontalchart-online.com; printed with permission of the Department of Periodontology, University of Bern, Switzerland). During the periodontal probing, six sites were examined per tooth (three vestibular sites: mesial, middle, and distal; and three lingual or palatal sites: mesial, middle, and distal). Additionally, the periapical radiographic series was taken on 14 radiographs as part of the periodontal diagnosis (14). Once this process was completed and the patient was found to need to undergo complete phase I periodontal treatment, each patient was questioned about their current situation of general dental sensitivity (without specifying any tooth) using Schiff Cold Air Sensitivity Scale (SCASS) (15). For the SCASS, initial sensitivity tests were carried out with a triple dental syringe with an air pressure between 45 and 60 psi, the adjacent teeth being relatively isolated using cotton gauze to avoid direct air contact to
them. A constant air pressure was maintained in the teeth to be analyzed (one tooth per patient), perpendicular to the longitudinal axis of the tooth for 5 s and at a distance of 3-5mm. According to the SCASS the patient is classified into one of the following categories, 0: without response, 1: responded without requiring stimulus discontinuation, 2: responded and requested discontinuation or moved from stimulus, 3: responded, considered stimulus painful. Using this scale, we classified the subjects as 0 being the absence of pain, 1 mild, 2 moderate and 3 intense.

All patients who recorded a pain score of 0 according to the SCASS continued in the study and underwent initial phase conventional periodontal therapy, which involved dental prophylaxis and dental plaque check (staining with dental plaque-revealing pigment and teaching of brushing technique); the removal of supragingival calculus with ultrasound (Bobcat, Densply Sirona, Charlotte, NC, USA); and surface polishing with a rubber cup and abrasive paste (Proxyt, Ivodent Vivadent, Schaan, Liechtenstein). At 8 days after the initial prophylaxis, SRP treatments were performed on each individual, at sites with a probing depth equal to or greater than 4mm using McCall or Gracey type curettes (Hu-Friedy, Chicago, IL, USA). Once completed, the SRP sessions were followed by a 30-day waiting period for healing and reinsertion of the periodontal tissues. Next, a periodontal reassessment was performed following the inclusion criteria previously proposed, and patients who reported post-SRP tooth sensitivity in at least one tooth were retained in the study.

Subsequently, the tooth to which the patient attributed the greatest sensitivity was selected for analysis; if, for some reason, the patient did not identify a specific tooth, but rather a region/zone/quadrant, then the one with the highest gingival recession measurement was selected as the study tooth. Once the post-SRP sensitivity measure (T0) was obtained, all patients were asked to brush their teeth three times a day using a commercially available low-fluoride toothpaste (Colgate Total 12, Colgate-Palmolive, New York, NY, USA), which was provided to the patient, since such toothpastes does not contain any compound that interferes with the study. Likewise, the patients were instructed to avoid the use of any desensitizing product that could interfere with the study. Study subjects were given a bottle of IMP+ED-based mouthwash (Enamelin®). The recommended use of this agent was explained to all patients. These instructions were as follows: Use the mouthwash 30min after brushing. The rinse must be placed in a horizontal position for 5min before using it so that the sediments can be properly homogenized. Once homogenized, the patient must rinse their mouth with 20mL of the undiluted solution for 1 min. The patients were instructed to avoid rinsing with water after rinsing with the solution. They were also instructed to avoid consuming food and/or beverages for the next 30min after rinsing. The patients were advised to repeat the aforementioned procedure thrice a day (every 8 hours) for 30 days.

At the end of the 30 days of treatment with the mouthwash, each individual was reassessed, carrying out the relative isolate protocol, using continuous air pressure on the same tooth previously observed in T0, and the results of the post-treatment SCASS (T1) were recorded.

**STATISTICAL ANALYSIS**

For statistical evaluation of the change of pain condition, patients with an index of 0 or 1 from the SCASS were classified as mild. Using the SPSS version 20, a McNemar-Bowker test was used to compare the correlated proportions between the levels of dental hypersensitivity before (T0) and
after (T1) the treatment with the IMP+ED-based mouthwash (p<0.05).

The treatment effect size on patients was calculated using the procedure recommended for contingency tables in the family of Chi-square tests included in the G* Power package (16).

RESULTS

The initial sample consisted of 55 patients, of whom 8 were excluded from the study due to a pain grade of 1 or greater according to the SCASS (n=5) or due to the impossibility of performing a monthly reassessment of pain (n=3). This resulted in a final sample size of 47 patients, with 23% (n=11) being men and 77% (n=36) women. The included patient population had an age range of 29-66 years and a mean age of 49.95+/−10.57 years. The frequency distribution of the teeth studied for each patient is reported in Table 1.

After the SRP therapy (T0), all the sample members reported pain distributed in the following manner: 12.8% were mild, 27.6% moderate, and 59.6% intense (Figure 1). At one month since treatment and with the use of the IMP+ED-based mouthwash (T1), the distribution of pain levels changed to 83% mild, 12.8% moderate, and 4.3% intense (Figure 1), this change was statistically significant (p<0.001) (Table 2). Finally, the treatment effect size on patients was: w=0.27 (moderate effect).

Table 1. Frequencies and percentages of teeth used in the study.

| First Quadrant | | | | | |
|----------------|---|---|---|---|---|
| Tooth          | 1.1 | 1.2 | 1.3 | 1.4 | 1.5 | 1.6 |
| Frequency (n)  | 5  | 2  | 1  | 3  | 0  | 2  |
| Percentage (%) | 10.6 | 4.3 | 2.1 | 6.4 | 0  | 4.3 |

| Second Quadrant | | | | | |
|-----------------|---|---|---|---|---|
| Tooth           | 2.1 | 2.2 | 2.3 | 2.4 | 2.5 | 2.6 |
| Frequency (n)   | 5  | 1  | 4  | 1  | 0  | 2  |
| Percentage (%)  | 10.6 | 2.1 | 8.5 | 2.1 | 0  | 4.3 |

| Third Quadrant  | | | | | |
|-----------------|---|---|---|---|---|
| Tooth           | 3.1 | 3.2 | 3.3 | 3.4 | 3.5 | 3.6 |
| Frequency (n)   | 7  | 2  | 0  | 1  | 1  | 1  |
| Percentage (%)  | 14.9 | 4.3 | 0  | 2.1| 2.1 | 2.1 |

| Fourth Quadrant | | | | | |
|-----------------|---|---|---|---|---|
| Tooth           | 4.1 | 4.2 | 4.3 | 4.4 | 4.5 | 4.6 |
| Frequency (n)   | 4  | 1  | 1  | 1  | 0  | 2  |
| Percentage (%)  | 8.5 | 2.1 | 2.1 | 2.1 | 0  | 4.3 |

Table 2. Result of the McNemar-Bowker test comparing the correlated proportions between the levels of dental hypersensitivity before (T0) and after (T1) the treatment with the IMP+ED-based mouthwash.

|     | T0 | T1          |               |               |              | p       |
|-----|----|-------------|---------------|---------------|--------------|---------|
|     | Mild | n | % | Moderate | n | % | Intense | n | % | Total | n | % |        |
| Mild | 6   | 100 |   | 0 | 0 |   | 0 | 0 |   | 6 | 12.8 | < 0.001* |
| Moderate | 12 | 92.3 | 1 | 7.7 | 1 | 7.7 | 2 | 7.1 | 15 | 27.6 |
| Intense | 21 | 75 | 5 | 17.9 | 2 | 7.1 | 28 | 59.6 |
A large number of patients report DH after periodontal treatment, which prevents them from maintaining adequate oral hygiene (17). Madhurkar et al. (2017), reported that individuals with periodontitis have a higher prevalence of hypersensitivity after periodontal treatment, especially SRP treatment (18).

The present study demonstrated that the use of IMP+ED-based mouthwash after completing SRP therapy effectively reduces painful responses to the exposure of the root surface to the oral environment. The results showed that, after SRP treatment, all patients presented some level of dental sensitivity (59.6% intense); however, one month after using the IMP+ED-based mouthwash, there was a significant reduction in pain levels (83% within the mild category).

Few studies have been conducted to examine the efficacy of desensitizing agents in mouthwash formulations. An eight-week study conducted by Yates et al. (1998) compared the desensitizing efficacy of a mouthwash containing 2% potassium citrate, fluoride, and cetylpyridinium chloride in contrast to a control mouthwash containing only fluoride. There was no significant difference in sensitivity scores after using the mouthwash (19). Additionally, in a six-week study conducted by Gillam (1996), the efficacy of a 3% fluoride mouthwash, which also contained potassium nitrate, was compared with that of a control mouthwash that only contained fluoride (20). In terms of treatment effects, pain measurement after exposure to thermal and tactile stimuli showed that a mouthwash containing potassium nitrate performed significantly better than a control mouthwash in improving these sensitivity scores. Another study, conducted by Pereira and Chava (2001), showed that no such clear advantage existed when using a mouthwash with potassium ions: the group of patients that used a mouthwash with potassium ions obtained significantly lower scores of air blast pain at six weeks, but no significant difference at two weeks (21). Taken together, these studies do not provide sufficient evidence to suggest that mouthwashes containing potassium ions are effective in reducing pain caused by tooth sensitivity. The results of both studies contrast with the findings of the present study since statistically significant differences were observed between the two periods of time in which the degree of pain experienced by the patient was evaluated.

Most of the mouthwashes used to reduce DH contain an active ingredient in addition to fluoride or calcium. In 2013, Hu et al. reported a significant reduction in DH, after continuous use for eight weeks, of rinses with 0.8% arginine, pyrophosphates, and 0.05% sodium fluoride in an alcohol-free mouthwash (22). These results are comparable with a study carried out by Schiff et al. (2011), who compared the desensitizing effect between toothpastes containing 8% arginine, calcium carbonate, and fluoride (Colgate Pro Alivio®) and those containing 8% arginine, 8% acetate strontium, and fluoride (Sensodyne Rapid Relief®). The results of these studies indicated that the use of Colgate Pro Alivio® toothpaste after eight weeks produced a superior desensitizing effect compared to the use of Sensodyne Rapid Alivio® toothpaste during the same period of time. Critically, when
comparing the present study with the two previous ones, it should be noted that the desensitizing effects after using the IMP+ED-based mouthwash manifested in half the time that was previously reported, since, according to the indications from the manufacturer, this mouthwash is only used for 4 weeks during the active phase. The efficacy of IMP+ED-based mouthwash may be related to its high remineralizing potential due to the addition of enamel-derived proteins (23).

Iacob and Veis (2008), showed that enamel-derived proteins (24), such as the included in the IMP+ED-based mouthwash, have the ability to drive the formation of cementum, periodontal ligament, and dentin in vitro, when placed in cells from rodent embryo dental follicles. Various studies agree with this finding (25,26), and accordingly, the regenerative potential of these proteins has been exploited in periodontal surgery. Currently, the exact mechanisms by which this process occurs remain unclear. However, the present study results supported that these enamel-derived proteins may play a role in the process of dentin remineralization, triggering a significant reduction in hypersensitivity.

The limitations of the present study include the small sample size, the lack of randomization and a control group, and the presence of possible biases due to uncontrolled variables, such as the influence of gingival recessions in the evolution of dentin sensitivity. Despite these limitations, it can be concluded that clinically, a mouthwash based on ionized calcium phosphates and derivatives of the protein enamelin is effective in treating DH, and its use is recommended for desensitizing purposes. Finally, given the treatment effect size on patients ($w=0.27$), it was calculated that 150 patients would be required to have a statistical power (Beta error) of 0.8. Statistical power with 47 patients, alpha error of 0.05 and 3 degrees of freedom is 0.31 (Beta error). Therefore, although the treatment effect size on patients is adequate, the probability of committing type 2 error can be improved significantly, increasing the sample size and controlling for some additional variables in the experiment.

CONCLUSIONS

Mouthwashes based on ionized calcium phosphates and derivatives of the protein enamelin produce a positive effect in reducing painful responses caused by exposure of the dentin tubules to the oral environment after SRP treatment.

CONFLICTS OF INTEREST

The manufacturer provided material support for this research (Operating System Select SA de CV). Operating System Select SA de CV did not participate in any part of the research development, and the results of this study have not been shared with them.

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REFERENCES

1. Salazar D., Nakouzi J., Salazar P.D., Nakouzi M.J. Clinical Evaluation Fluoride Varnish in the Management of Dentin Hypersensitivity. Int J Odontostomat. 2017; 11 (1): 41-6.
2. Romero-Amaro I.M., Escalona L., Acevedo A.M. Teorías y factores etiológicos involucrados en la hipersensibilidad dentinaria. Acta Odontol Venez. 2009; 47 (1): 260-9.
3. Shiau H.J. Dentin hypersensitivity. J Evid Based Dent Pract. 2012;12 (3 Suppl): 220-8.
4. Collaert B., Fischer C. Dentine hypersensitivity: a review. Dent Traumatol. 1991; 7 (4): 145-52.
5. Humphreys S.M. Henry James’s revisions for “the ambassadors.” Notes Queries. 1954; 199: 397-9.
6. Rane P., Pujari S., Patel P., Gandhewar M., Madria K., Dhume S. Epidemiological Study to Evaluate the Prevalence of Dentine Hypersensitivity among Patients. J Int oral Heal. 2013; 5 (5): 15-9.
7. Nanjundasetty J.K., Ashrafulla M. Efficacy of desensitizing agents on postoperative sensitivity following an in-office vital tooth bleaching: A randomized controlled clinical trial. J Conserv Dent. 2016; 19 (3): 207-11.
8. Orchardson R., Gillam D.G. Managing dentin hypersensitivity. J Am Dent Assoc. 2006; 137 (7): 990-8.
9. Moreau J.L., Sun L., Chow L.C., Xu H.H.K. Mechanical and acid neutralizing properties and bacteria inhibition of amorphous calcium phosphate dental nanocomposite. J Biomed Mater Res Part B Appl Biomater. 2011; 98B (1): 80-8.
10. Sharda S., Prasad K.V.V., Shetty P.J., Nikhil K. Effectiveness of Desensitizing Dentifrice and Mouthwash on Dentin Hypersensitivity and Tooth Remineralization. Contemp Clin Dent. 2018; 9 (3): 415-20.
11. Yates R., West N., Addy M., Marlow I. The effects of a potassium citrate, cetylpyridinium chloride, sodium fluoride mouthrinse on dentine hypersensitivity, plaque and gingivitis. J Clin Periodontol. 1998; 25 (10): 813-20.
12. Hurtado P., Tobar-Tosse F., Osorio J., Orozco L., Moreno F. Amelogenesis imperfecta: revisión de la literatura. Rev Estomatol. 2015; 23 (1): 32-41.
13. Banfield N., Addy M. Dentine hypersensitivity: Development and evaluation of a model in situ to study tubule patency. J Clin Periodontol. 2004; 31 (5): 325-35.
14. Caton J.G., Armitage G., Berglundh T., Chapple I.L.C., Jepsen S., Konman K.S., et al. A new classification scheme for periodontal and peri-implant diseases and conditions-Introduction and key changes from the 1999 classification. J Periodontol. 2018; 89 (S1): 1-8.
15. Schiff T., Dotson M., Cohen S., De Vizio W., McCool J., Volpe A. Efficacy of a dentifrice containing potassium nitrate, soluble pyrophosphate, PVM/MA copolymer, and sodium fluoride on al hypersensitivity: a twelve-week clinical study. Journal of Clinical Dentistry. 1994; 5: 87-92.
16. Faul F., Erdfelder E., Lang A.G., Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007; 39 (2): 175-91.
17. Pepelassi E., Rahiotis C., Peponi E., Kakaboura A., Vrotsos I. Effectiveness of an in-office arginine-calcium carbonate paste on dentine hypersensitivity in periodontitis patients: A double-blind, randomized controlled trial. J Clin Periodontol. 2015; 42 (1): 37-45.
18. Madhurkar J.G., Bhat P.R., Acharya A.B., Thakur S.L., Trasad V.A. Efficacy of milk
as a desensitizing agent for the treatment of sensitivity following scaling and root planing. Contemp Clin Dent. 2017; 8 (2): 231-5.

19. Yales R., West N., Addy M., Marlow I. The effects of a potassium citrate, cetlypyridinium chloride, sodium fluoride mouthrinse on dentine hypersensitivity, plaque and gingivitis. A placebo-controlled study. J Clin Periodontol. 1998; 25 (10): 813-20.

20. Gillam D.G. Efficacy of a potassium nitrate mouthwash in alleviating cervical dentine sensitivity (CDS). J Clin Periodontol. 1996; 23 (11): 993-7.

21. Pereira R., Chava V.K. Efficacy of a 3% Potassium Nitrate Desensitizing Mouthwash in the Treatment of Dentinal Hypersensitivity. J Periodontol. 2001; 72 (12): 1720-5.

22. Hu D., Stewart B., Mello S., Arvanitidou L., Panagakos F., De Vizio W., et al. Efficacy of a mouthwash containing 0.8% arginine, PVM/MA copolymer, pyrophosphates, and 0.05% sodium fluoride compared to a negative control mouthwash on dentin hypersensitivity reduction. A randomized clinical trial. J Dent. 2013; 41 (Suppl. 1): 26-33.

23. Schiff T., Mateo L.R., Delgado E., Cummins D., Zhang Y.P., Devizio W. Clinical efficacy in reducing dentin hypersensitivity of a dentifrice containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride compared to a dentifrice containing 8% strontium acetate and 1040 ppm fluoride under consumer usage conditions before. J Clin Dent. 2011; 22 (4): 128-38.

24. Iacob S., Veis A. Identification of the functional activity of the [A-4] amelogenin gene splice product in newborn mouse ameloblasts. Bone. 2008; 42 (6): 1072-9.

25. Papagerakis P., Ibarra J.M., Inozentseva N., DenBesten P., MacDougall M. Mouse amelogenin exons 8 and 9: Sequence analysis and protein distribution. J Dent Res. 2005; 84 (7): 613-7.

26. Fincham A.G., Hu Y., Lau E.C., Slavkin H.C., Snead M.L. Amelogenin post-secretory processing during biomineralization in the postnatal mouse molar tooth. Arch Oral Biol. 1991; 36 (4): 305-17.