Journal of Oral Biology and Craniofacial Research 10 (2020) 727–732

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Original Article

An *in vitro* analysis of the effect of adjunctive use of ozonated oil with a desensitizing agent on dentinal tubule occlusion

H.R. Veena[a,*], C. Afigith Mathew[a], Riya Achamma Daniel[a], P. Shubha[b], R. Sreeparvathy[a], Neha Pradhan[a]

[a] Department of Periodontics, K. L. E. Society's Institute of Dental Sciences, Bangalore, 560022, India
[b] Department of Material Science, Mangalagangotri, Mangalore University, Mangalore, 574199, India

ARTICLE INFO

Keywords:
Ozone
Dentin desensitizing agents
Dentin sensitivity
Arginine

ABSTRACT

Introduction: Dentinal hypersensitivity (DH), is a commonly encountered clinical problem, the management of which is by two main approaches that involve blockage of nerve activity and tubular occlusion. Desensitizing agents containing arginine and calcium carbonate have emerged effective in occluding tubules. Ozone removes the smear layer and opens up the dentinal tubules for easy penetration of these desensitizing agents.
Objective: To comparatively evaluate the effect of ozonated oil on the patency and occlusion of dentinal tubules with and without adjunctive application of a desensitizing agent containing arginine.
Materials and methods: 80 dentin specimens that were distributed into 4 groups as Group 1 (control), Group 2 (ozonated oil), Group 3 (desensitizing agent) and Group 4 (desensitizing agent following the application of ozonated oil), underwent an acid challenge. Scanning electron microscopic (SEM) images of the dentin specimens were obtained prior to any treatment, after the application of the therapeutic agents and after acid challenge and mean tubule occlusion scores were recorded in each case employing a suitable scoring system. The statistical analysis employed One-way ANOVA test followed by Tukey's Post hoc test and Student Paired *t*-test for intergroup and intragroup comparisons of the mean tubule occlusion scores respectively.
Results: Group 2, treated with ozonated oil alone showed a statistically significant increase in the number of open dentinal tubules. Group 4 showed more compact deposits of desensitizing agent and more densely occluded tubules as compared to Group 3 which was more retained in the former even after acid challenge.
Conclusion: Adjunctive application of the desensitizing agent containing arginine, with ozonated oil has a synergistic effect, where the latter causes opening of dentinal tubules allowing more compact penetration of the former and thus may be a potentially more effective treatment approach in the management of DH.

1. Introduction

Dentinal hypersensitivity (DH), a frequently encountered dental complaint in adults, is characterized by short, sharp pain due to exposure of dentinal tubules to the external environment as a result of enamel or cementum loss which cannot be ascribed to any other dental defect or pathology. Dentinal tubules are pivotal in carrying stimuli and irritants to the pulp. The hydrodynamic theory of DH states that the dentinal tubules exposed to the oral environment, under the presence of certain stimuli cause displacement of fluids within the tubules which indirectly stimulates pulpal nerve receptors causing pain.[1]

Currently, blockage of nerve activity and tubular occlusion are the two main approaches used for management of DH.[2] Tubular occlusion can be achieved by mechanically occluding them by employing a physical or chemical agent that plugs the open tubules. Several dentifrices that incorporate desensitizing agents such as fluoride, calcium hydroxide and potassium nitrate have been prescribed to achieve relief from DH. Technological innovation recently led to the development of a novel and effective therapeutic approach against DH, which is based on arginine and calcium carbonate. Arginine, a naturally occurring amino acid in saliva, acts synergistically with calcium carbonate and phosphate and creates an occlusive adhesive (plug-in) in the dentinal tubules, thus preventing fluid flow.[3] Although various chemicals are expected to occlude the exposed tubules and provide instant relief for DH,

* Corresponding author. Department of Periodontics, K. L. E. Society's Institute of Dental Sciences, No: 20, Tumkur road, Yeshwanthpur, Bangalore, Karnataka, 560022, India.
E-mail addresses: drveenahr@gmail.com (H.R. Veena), afigithmathewc2010@gmail.com (C. Afigith Mathew), riyaad.rd@gmail.com (R.A. Daniel), shubhadruti@yahoo.com (P. Shubha), nairsreeparvathy61@gmail.com (R. Sreeparvathy), pradhanneha92@yahoo.com (N. Pradhan).

https://doi.org/10.1016/j.jobcr.2020.10.001
Received 9 June 2020; Received in revised form 29 September 2020; Accepted 1 October 2020
Available online 07 October 2020

the effect is not long lasting.[4,5] Hence, management of DH should aim at providing long lasting effect.

Ozone, first discovered in 1840 by the German chemist Christian Friedrich Schonbein, is an allotropic form of oxygen which exists in nature as a gaseous molecule comprising of three oxygen atoms. It is a powerful oxidizing agent which effectively kills bacteria, fungi, viruses and parasites. Medical grade ozone is in therapeutic practice for over the last 100 years. Its immunostimulatory, analgesic, detoxifying, antimicrobial, biosynthetic and bioenergetic properties, make it unique for several applications in the field of dentistry. Ozone therapy, uses oxygen/ozone administered via gas or dissolved in water or oil base to obtain a therapeutic benefit, and has been considered as a versatile, bio-oxidative therapy found to be an effective treatment modality for DH. Being non invasive, it also enhances patient compliance.[6]

Lately, herbal extracts have also paved their way into the field of dentistry in a wide range of arenas. The test material used in our research is constituted of several oils of plant origin with therapeutic effects like Olive oil, Hemp seed oil, Stevia and Natural cherry oil.

With a null hypothesis that, the combined use of ozone and desensitizing agent results in no improvement in the percentage of tubular occlusion as compared to the use of desensitizing agent alone, this study aimed to comparatively evaluate the effect of application of ozonated oil with and without an adjunctive desensitizing agent containing arginine on the patency and occlusion of dentinal tubules in vitro.

2. Materials and methods

The study was carried out in the Department of Periodontics of a tertiary dental hospital and research institute in Southern India. The sample specimens for the study included human supernumerary teeth, impacted teeth or teeth indicated for extraction for orthodontic purposes. The sample size for the study was estimated using the software G Power v. 3.1.9.2. Considering the effect size to be measured at 0.80, power of the study at 80% and the margin of the α-error at 5%, the sample size was calculated as 20 for each of the 4 groups, contributing a total of 80 dentin specimens. The teeth specimens extracted under local anesthesia by a registered oral surgeon were collected after obtaining clearance from the institutional ethics committee. Extracted human premolars and molars free of dental caries on both the crown and root surfaces and devoid of abrasion or erosion were selected. Teeth with a history of periodontal therapy within 6 months prior to extraction, teeth extracted due to periodontal disease and endodontically treated teeth were excluded from the study.

2.1. Preparation of dentin specimens[7]

Freshly extracted human teeth specimens fulfilling the inclusion and exclusion criteria were cleaned with hydrogen peroxide (6% w/v) to remove debris and blood and stored in 10% formalin until further use. A double sided diamond disk operated on a micro-motor with water-cooled mechanism (Aseptico-M4B-27755,USA) was used in conjunction with a straight handpiece (Uniq –Kavo, Germany) to prepare approximately 1.5 mm thick cross-sectional dentin slices from the crown portion of the tooth. 80 dentin discs were prepared and polished for 30 s on one side using 600-grit silicon carbide paper to create a uniform surface. The polished specimens were later sonicated at 30 kHz for 60 s in deionized water contained in a polycarbonate tube to remove the polishing abrasive. The dentin specimens were thoroughly rinsed with deionized water followed by the placement in a fresh polycarbonate tube containing 37% *ortho*-phosphoric acid solution and sonicated for 30 s. After etching by acid, the specimens were rinsed with Milli-Q water and stored in phosphate buffered saline (PBS) at a pH of 7.4.

2.2. Experimental design (Fig. 1)

A total of 80 dentin specimens were prepared and equally divided into four groups by simple randomization following a computer generated table.

Group 1 (control) – 20 prepared specimens not treated with any therapeutic agent.

Group 2–20 prepared specimens treated with ozonated oil (Pur O3 tooth and gum support constituted by organic *Olea europaea* oil, organic *Cannabis sativa* seed oil, organic *Stevia rebaudiana*, organic natural cherry and ozone).

Group 3–20 prepared specimens treated with desensitizing agent (Colgate sensitive pro-relief containing 8% arginine, calcium carbonate and 1450 ppm fluoride as sodium monofluorophosphate).

Group 4–20 prepared specimens treated with ozonated oil followed by the application of desensitizing agent.

2.3. Sample preparation for scanning electron microscopy

The dentin discs were mounted on stubs and coated with gold sputter for subsequent SEM analysis. Then images of SEM were recorded at 2000×, 4000× and 15000× magnifications.

The procedure was repeated with each of the dentin disc following the respective treatment protocol and following acid treatment.

2.4. Application of ozonated oil and desenstizing agent

Dentin discs placed on a glass slide with polished side facing upwards were secured with a double sided carbon tape. The specimens in Group 2 were wetted with PBS and brushed with ozonated oil using a powered toothbrush (Colgate 360° whole mouth clean) operated at 10,914 oscillations/min for 120 s. In Group 3, specimens were wetted with PBS and brushed with desensitizing agent as mentioned above. In Group 4, specimens were wetted with PBS and brushed with ozonated oil followed by desensitizing agent as described before. The specimens were then gently rinsed with Milli-Q water to ensure removal of excess dentifrice from the surface and stored in vacuum until SEM analysis and further use.[7]

The force of brushing was standardised by employing a customised holder made of silicone putty while the toothbrush was in use.

2.5. Acid challenge

Dentin specimens from Group 1 and treated dentin specimens from Groups 2, 3 and 4 were further immersed in 4 mL of 6% citric acid (pH 2) in a 35 mm petridish. The samples were left undisturbed for 60 s followed by rinsing with PBS and was thus prepared for subsequent SEM analysis.

2.6. Evaluation of tubule occlusion

The SEM images were assessed (on a scoring scale of 1–5) for the extent of dentinal tubule occlusion. The scoring was done as follows.[7]

Score 1: Occluded (100% of tubules occluded).
Score 2: Mostly occluded (75% of tubules occluded).
Score 3: Equally occluded/unoccluded (50% of tubules occluded).
Score 4: Mostly unoccluded (25% of tubules occluded).
Score 5: Unoccluded (0% no tubule occlusion).

The SEM analysis of dentinal tubule occlusion in Group 1 (Control group) was done twice i. e before and after acid challenge. The SEM analysis of the remaining 60 specimens was done thrice, i. e.

- Prior to any treatment (Baseline)
- Immediately following the respective treatment protocol (Before acid treatment)
- Immediately following acid treatment.

The analysis was performed by three independent blinded reviewers for each specimen at all times, so as to minimise subjective bias. The mean score of tubule occlusion was calculated for each specimen before and after acid challenge.

2.7. Statistical analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp, was used to perform statistical analyses. Descriptive analysis included the tubule occlusion scores in terms of mean and standard deviation (SD). The data followed a normal distribution. Hence, all relevant inferential analyses were performed using parametric tests. The level of significance i. e the p value was set at < 0.05. Comparison of mean dentinal tubule occlusion scores between different groups both before and after acid challenge was done using One-way ANOVA test. Multiple comparisons of mean difference in tubule occlusion scores before and after acid challenge between groups was done using Tukey's Post hoc test. Comparison of mean dentinal tubule occlusion scores before and after acid challenge in each group was done using Student Paired *t*-test.

3. Results

The prepared dentin discs were analyzed using SEM and it was observed that the natural architecture was retained after etching (Fig. 2a).

3.1. Before acid challenge

Dentin discs exposed to ozonated oil (Group 2) showed a significant number of open tubules (Fig. 2b). Groups 3 and 4 revealed relatively greater tubular occlusion with precipitates of desensitizing agent and fewer patent dentinal tubules (Fig. 2c and d). The SEM images of Group 4 recorded at higher magnifications (15000×), showed more compact precipitation of desensitizing agent in the peritubular dentin and deposition into the tubules resulting in greater degree of tubular occlusion compared to Group 3. There was a statistically significant difference between the mean tubule occlusion scores of various groups with a $p < 0.001$ (Table 1). Group 2 showed significantly higher mean tubule occlusion score compared to Groups 3 and 4 ($p < 0.001$) indicating close to 0% tubule occlusion while Group 3 showed significantly higher mean tubule occlusion compared to Group 4 ($p < 0.001$) (Table 2).

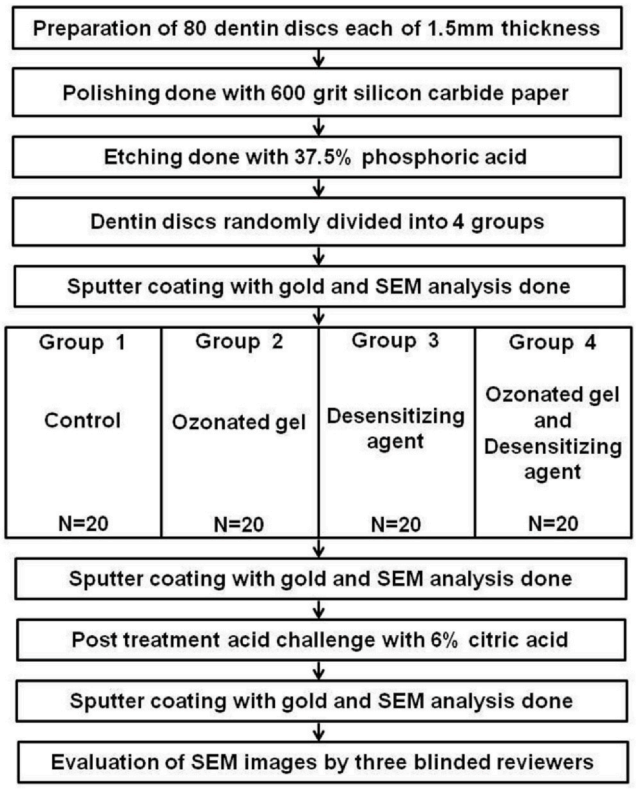

Fig. 1. Summary of the experimental design.

3.2. After acid challenge

Following acid challenge there was significant opening of dentinal tubules of the treated samples in all groups. Both Groups 1 and 2 revealed tubule occlusion scores on the higher end and the latter showed a significant increase in tubule occlusion score after acid challenge with a mean difference of -0.25 ($p = 0.02$) (Table 3). The tubule occlusion scores in Groups 3 and 4 were towards the lower end (Table 1) and were significantly lower than Group 2 ($p < 0.001$ and $p < 0.001$) (Table 2). Compared to Group 3 ($p < 0.001$), Group 4 ($p < 0.01$) had less significant change in tubule occlusion score (Table 3). The results showed that even after acid challenge, Groups 3 and 4 showed retained tubule occlusion and Group 4 revealed more retained tubule occlusion. SEM images recorded at higher magnifications (15000×), revealed more compact precipitation and dense depositions of desensitizing agent in Group 4 with relatively greater degree of tubular occlusion compared to Group 3 (Fig. 2e and f).

4. Discussion

The most widely accepted theory of DH, the hydrodynamic theory proposed by Brannstrom, correlates with the number of open dentin tubules, which may be the reason for the increased fluid movement that causes DH.[1] The results of previous research which employed SEM revealed that in comparison to the number of tubules in non sensitive dentin, the number of tubules in sensitive dentin is eight times that of the former.[8] The sensitive tubules present with a wider diameter as compared to non sensitive tubules.[9] Most agents clinically employed in the management of DH occlude these open dentinal tubules.

When a desensitizing paste containing arginine is applied to exposed dentin, the positively charged arginine and calcium carbonate, naturally found in saliva, are believed to work together and bind to the negatively charged dentin surface to deposit a plug within the dentinal tubules and form a protective layer on the dentin surface.[3] This plug consisting of arginine, calcium carbonate, phosphate and salivary glycoproteins, reaches a depth of 2 μm into the tubule.[10] In this study, both Groups 3 and 4 treated with arginine containing desensitizing agent showed greater percentage of occluded dentinal tubules. The lower scores of tubule occlusion after acid challenge in groups 3 and 4 compared to groups 1 and 2, suggested a retained tubular occlusion in these groups. This is in accordance to several clinical trials that showed immediate beneficial effects of arginine and calcium carbonate containing toothpastes which lasted up to 8 weeks following treatment; and in certain studies these effects lasted up to 24 weeks.[11–20]

Although the primary etiopathogenesis of DH is known to be the exposure of dentin by tooth surface wear, not all of the exposed dentin is sensitive. This is because some of the dentinal tubules are shielded by a layer of smear layer.[11] Ozone has been known to initiate the removal of this smear layer, open the dentinal tubules and widen them.[6] This study also revealed that group 2, treated with ozonated oil alone showed a significantly greater percentage of open dentinal tubules, which is in accordance with previous research work.[6] On applying a remineralizing agent, calcium and fluoride ions enter the dentinal tubules easily, readily and deeply, and completely and effectively plug the dentinal tubules opened by ozone, preventing the fluid exchange across these tubules.[6] The high magnification SEM images of this study too revealed a more compact precipitation of desensitizing agent in the

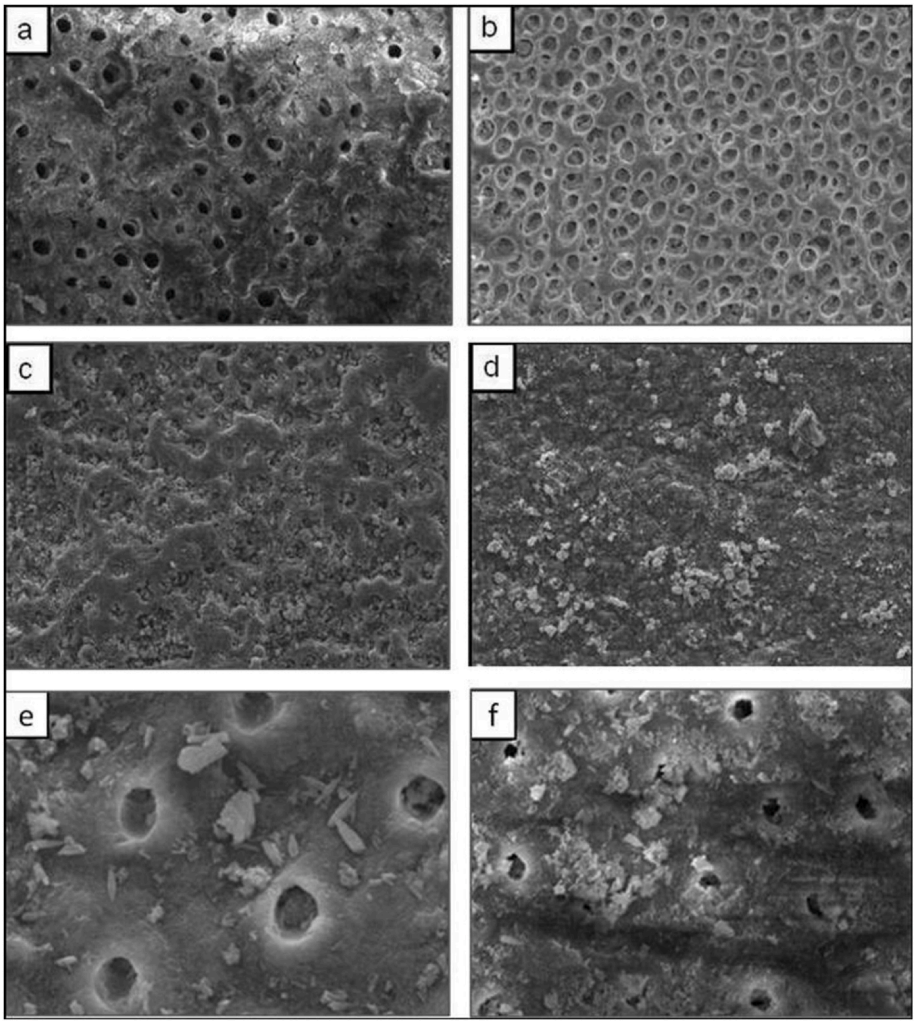

Fig. 2. Cross sectional SEM images of dentin surface morphology and tubular occlusion (a) Control group (before acid challenge) (b) Group employing ozonated oil (before acid challenge) (c) Group employing desensitizing agent (before acid challenge) (d) Group employing ozonated oil followed by desensitizing agent (before acid challenge) (e) Group employing desensitizing agent (following acid challenge) at 15000× magnification (f) Group employing ozonated oil followed by desensitizing agent (following acid challenge) at 15000× magnification.

Table 1
Comparison of mean dentinal tubule occlusion scores between different groups during using One-way ANOVA test.

Acid challenge	Groups	N	Mean	SD	Min	Max	*P*-value
Before acid challenge	Group 1	20	4.1	0.64	3	5	< 0.001*
	Group 2	20	4.55	0.69	3	5	
	Group 3	20	2.5	0.69	1	3	
	Group 4	20	1.55	0.61	1	3	
After acid challenge	Group 1	20	4.25	0.64	3	5	< 0.001*
	Group 2	20	4.8	0.41	4	5	
	Group 3	20	3.55	0.76	1	4	
	Group 4	20	1.85	0.67	1	3	

peritubular dentin and within the tubules resulting in more densely occluded tubules in group 4 compared to group 3, both before and after acid challenge. A more retained tubular occlusion was also observed in group 4 compared to group 3. It can thus be inferred from our in vitro study that the application of ozone followed by arginine containing desensitizing paste, has a synergistic effect, where the former causes opening of all dentinal tubules allowing more dense and compact deposition of desensitizing agents and thus providing a complete and more effective tubular occlusion irrespective of whether the tubule contributed to sensitivity or not. Thus, it may serve as a potential preventive strategy as well.

Phytomedicine or herbal medicine is considered an important alternative to allopathic medicine owing to its natural therapeutic benefits and minimal adverse effects.[21] The chief ingredient of our test agent, Olive oil (*Olea europaea*) along with Hemp seed oil and Natural cherry oil can form a film of lipids at the dentin surface which would act as a protective coating and increase the lipid content in the latter's outermost layer, thus hampering the diffusion of acids and mineral during demineralization.[22] Olive oil with its "sukshma" property allows its penetration even into minute channels.[23] Natural cherry oil, Stevia (*Stevia rebaudiana*) and Hemp seed oil (*Cannabis sativa*) contained in the ozonated oil have been identified to possess anti-inflammatory properties.[24–26] Natural cherry oil has also been found to possess antimicrobial, antibacterial and antifungal properties in addition to its antioxidant properties.[24] Stevia has also been found to possess antimicrobial properties, antibacterial properties, anti oxidant properties, antihyperglycemic and antidiabetic properties.[25] A synergistic action of these agents may help maintain homeostasis of the periodontal tissues adjacent to teeth. Stevia which has also been employed as a non calarogenic sweetener or flavouring agent, however, is found to be both anti-cariogenic as well as *anti*-periodontopathogenic.[27] The constituent ingredients of the ozonated oil employed in our study thus may have

Table 2
Multiple comparison of mean difference in dentinal tubule occlusion scores between groups using Tukey's Post hoc test.

Acid challenge	(I) Groups	(J) Groups	Mean diff. (I-J)	95% CI of the diff.		*P*-value
				Lower	Upper	
Before acid challenge	Group 1	Group 2	−0.45	−0.99	0.09	0.14
		Group 3	1.6	1.06	2.14	< 0.001*
		Group 4	2.55	2.01	3.09	< 0.001*
	Group 2	Group 3	2.05	1.51	2.59	< 0.001*
		Group 4	3	2.46	3.54	< 0.001*
	Group 3	Group 4	0.95	0.41	1.49	< 0.001*
After acid challenge	Group 1	Group 2	−0.55	−1.08	−0.02	0.04*
		Group 3	0.7	0.17	1.23	0.004*
		Group 4	2.4	1.87	2.93	< 0.001*
	Group 2	Group 3	1.25	0.72	1.78	< 0.001*
		Group 4	2.95	2.42	3.48	< 0.001*
	Group 3	Group 4	1.70	1.17	2.23	< 0.001*

Table 3
Comparison of mean dentinal tubule occlusion scores between before and after acid challenge in each group using Student Paired *t*-test.

Groups	Time	*N*	Mean	SD	Mean diff	*P*-value
Group 1	Before	20	4.1	0.64	−0.15	0.08
	After	20	4.25	0.64		
Group 2	Before	20	4.55	0.69	−0.25	0.02*
	After	20	4.8	0.41		
Group 3	Before	20	2.5	0.69	−1.05	< 0.001*
	After	20	3.55	0.76		
Group 4	Before	20	1.55	0.61	−0.3	0.01*
	After	20	1.85	0.67		

potential therapeutic effects.

Although, the therapeutic agents employed in this study are commercially available products to be used in the oral cavity, their applications may be limited due to their adverse effects. Ozone is neither a readily available nor a stable molecule and may be toxic at a concentration of 0.0007% per application.[28] An in vitro study observed that brushing with desensitizing paste containing 8% arginine and calcium carbonate resulted in slight surface roughness of human enamel although not statistically significant.[29] However, this study did not evaluate the adverse effects of the tested agents.

Although this study is the first of its kind to evaluate the effects of adjunctive use of a desensitizing paste containing arginine with ozone on dentinal tubule occlusion, future studies are required to clinically validate the probable superior and long lasting effect of this synergistic combination in the management of DH.

5. Conclusion

The adjunctive application of ozonated oil with a desensitizing agent caused more compact deposition of the particles of the latter compared to application of the desensitizing agent alone. A possible synergistic effect was noted, where the ozonated oil caused opening of dentinal tubules allowing more compact penetration of the desensitizing agent. With these observations, it may be reasonable to hypothesize that the adjunctive application of these two agents may provide appreciable clinical therapeutic benefit leading to effective management of dentinal hypersensitivity.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Declaration of competing interest

None.

References

1. Brännström M, Lindén LÅ, Åström A. The hydrodynamics of the dental tubule and of pulp fluid. *Caries Res.* 1967;1:310–317.
2. Trushkowsky RD, Oquendo A. Treatment of dentin hypersensitivity. *Dent Clin.* 2011;55:599–608.
3. Lavender SA, et al. Mode of action studies of a new desensitizing dentifrice containing 8.0% arginine, a high cleaning calcium carbonate system and 1450 ppm fluoride. *Am J Dent.* 2010;23 14A-9A.
4. Torwane NA, et al. Effect of two desensitizing agents in reducing dentin hypersensitivity: an in-vivo comparative clinical trial. *J Clin Diagn Res.* 2013;7:2042–2046.
5. Palazon MT, et al. Immediate and short-term effects of in-office desensitizing treatments for dentinal tubule occlusion. *Photomed Laser Surg.* 2013;31:274–282.
6. Tiwari S, Avinash A, Katiyar S, Iyer AA, Jain S. Dental applications of ozone therapy: a review of literature. *Saudi J Dent Res.* 2017;8:105–111.
7. Datey A, Thaha CA, Patil SR, Gopalan J, Chakravortty D. Enhancing the efficiency of desensitizing agents with shockwave treatment–a new paradigm in dentinal hypersensitivity management. *RSC Adv.* 2016;6:68973–68978.
8. Davari AR, Ataei E, Assarzadeh H. Dentin hypersensitivity: etiology, diagnosis and treatment; a literature review. *J Dent.* 2013;14:136–145.
9. Absi EG, Addy M, Adams D. Dentine hypersensitivity: a study of the patency of dentinal tubules in sensitive and non sensitive cervical dentine. *J Clin Periodontol.* 1987;14:280–284.
10. Panagakos F, Schiff T, Guignon A. Introducing pro-argin™-A breakthrough technology based upon arginine and calcium for in-office treatment of dentin hypersensitivity. *Am J Dent.* 2009;22:3A–7A.
11. Nathoo S, Delgado E, Zhang YP, DeVizio W, Cummins D, Mateo LR. Comparing the efficacy in providing instant relief of dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride relative to a benchmark desensitizing toothpaste containing 2% potassium ion and 1450 ppm fluoride, and to a control toothpaste with 1450 ppm fluoride: a three-day clinical study in New Jersey, USA. *J Clin Dent.* 2009;20:123–130.
12. Docimo R, et al. Comparing the efficacy in reducing dentin hypersensitivity of a new toothpaste containing 8.0% arginine, calcium carbonate, and 1450 ppm fluoride to a commercial sensitive toothpaste containing 2% potassium ion: an eight-week clinical study in Rome, Italy. *J Clin Dent.* 2009;20:137–143.
13. Que K, et al. Dentin hypersensitivity reduction of a new toothpaste containing 8.0% arginine and 1450 ppm fluoride: an 8-week clinical study on Chinese adults. *Am J Dent.* 2010;23:28A–35A.
14. Hamlin D, Mateo LR, Dibart S, Delgado E, Zhang YP, DeVizio W. Comparative efficacy of two treatment regimens combining in-office and at-home programs for dentin hypersensitivity relief: a 24-week clinical study. *Am J Dent.* 2012;25:146–152.
15. Boneta AR, et al. Efficacy in reducing dentine hypersensitivity of a regimen using a toothpaste containing 8% arginine and calcium carbonate, a mouthwash containing 0.8% arginine, pyrophosphate and PVM/MA copolymer and a toothbrush compared to potassium and negative control regimens: an eight-week randomized clinical trial. *J Dent.* 2013;41:S42–S49.
16. Kapferer I, Pflug C, Kisielewsky I, Giesinger J, Beier US, Dumfahrt H. Instant dentin hypersensitivity relief of a single topical application of an in-office desensitizing paste containing 8% arginine and calcium carbonate: a split-mouth, randomized-controlled study. *Acta Odontol Scand.* 2013;71:994–999.
17. Franca IL, Sallum EA, Do HV, Casati MZ, Sallum AW, Stewart B. Efficacy of a combined in-office/home-use desensitizing system containing 8% arginine and calcium carbonate in reducing dentin hypersensitivity: an 8-week randomized clinical study. *Am J Dent.* 2015;28:45–50.
18. 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;42:37–45.
19. Samuel SR, Khatri SG, Acharya S, Patil ST. Evaluation of instant desensitization after a single topical application over 30 days: a randomized trial. *Am J Dent.* 2015;60:336–342.
20. Hirsiger C, et al. Efficacy of 8% arginine on dentin hypersensitivity: a multicenter clinical trial in 273 patients over 24 weeks. *J Dent.* 2019;83:1–6.
21. Bhatt A. Phytopharmaceuticals: a new drug class regulated in India. *Perspect Clin Res.* 2016;7:59–61.
22. Wiegand A, Gutsche M, Attin T. Effect of olive oil and an olive-oil-containing fluoridated mouthrinse on enamel and dentin erosion in vitro. *Acta Odontol Scand.* 2007;65:357–361.
23. Kulkarni M, Dubewar AP, Kutte AP. A conceptual review of properties of olive oil as per Ayurveda with special reference to Anukta Dravya. *J Sci Innov Res.* 2016;5:152–155.
24. Victoria FN, et al. Essential oil of the leaves of Eugenia uniflora L.: antioxidant and antimicrobial properties. *Food Chem Toxicol.* 2012;50:2668–2674.
25. Singh A, Agrawal N, Gupta AK. A 'sweet shrub'stevia: more than a sweetener. *IJOD.* 2014;2:1–4.
26. Acharya R. Vijaya (Cannabis sativa Linn.) and its therapeutic importance in Ayurveda; a review. *JDRAS.* 2015;1:1–12.
27. Contreras MS. Anticariogenic properties and effects on periodontal structures of stevia rebaudiana bertoni. narrative review. *J Oral Res.* 2013;2:158–166.
28. Gupta S, Deepa D. Applications of ozone therapy in dentistry. *J Oral Res Rev.* 2016;8:86–91 1.
29. Garcia-Godoy FR, Garcia-Godoy A, Garcia-Godoy C. Effect of a desensitizing paste containing 8% arginine and calcium carbonate on the surface roughness of dental materials and human dental enamel. *Am J Dent.* 2009;22:21A–24A.