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ORIGINAL ARTICLE

Normal Saline or Sodium Hypochlorite Irrigation for Vital Pulp Therapy? A Non-Inferiority Randomised Controlled Trial

Hataichanok MACHAREONSAP,[1] Papimon CHOMPU-INWAI,[1] Nattakan CHAIPATTANAWAN,[1] Chanika MANMONTRI,[1] Areerat NIRUNSITTIRAT,[2] Phichayut PHINYO[3,4,5]

[1]Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Chiang Mai University Faculty of Dentistry, Chiang Mai, Thailand
[2]Division of Community Dentistry, Department of Family and Community Dentistry, Chiang Mai University Faculty of Dentistry, Chiang Mai, Thailand
[3]Department of Family Medicine, Chiang Mai University Faculty of Medicine, Chiang Mai, Thailand
[4]Center for Clinical Epidemiology and Clinical Statistics, Chiang Mai University Faculty of Medicine, Chiang Mai, Thailand
[5]Musculoskeletal Science and Translational Research (MSTR) Cluster, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT

Objective: To compare treatment success and discolouration of the permanent posterior teeth treated with vital pulp therapy (VPT) using mineral trioxide aggregate (MTA), irrigated with sodium hypochlorite (NaOCl) or normal saline solution.

Methods: A randomised non-inferiority trial was conducted. One hundred and twenty-five teeth that met the inclusion criteria were randomised to each irrigant, 65 in the NaOCl and 60 in the NSS groups. ProRoot® MTA was used as a pulp dressing material in both groups and teeth were followed for 12 months. The primary outcome was the success of VPT; requiring both clinical and radiographic success to be considered as success. The hypothesis of the primary outcome of this study was that the absolute difference of VPT success in the NSS group was not worse than that in the NaOCl group, by a margin of 5%. The hypothesis of the secondary outcome was that the NSS group would have a lower percentage of discolouration compared to the NaOCl group.

Results: Using a per protocol analysis, the absolute difference of VPT success between the NSS and NaOCl groups was 2.08% (95% CI: -1.95, 6.1). Perceptible grey discolourations were 80% and 63% in NaOCl and NSS groups (difference -17%; 95% CI: -40.0, 6.2; p=0.15).

Conclusion: For MTA-VPT procedure, irrigation with NSS was not worse than that with NaOCl. However, NSS did not lower the percentage of discolouration as discolouration was found in both irrigant groups.

Keywords: Discolouration, mineral trioxide aggregate, saline solution, sodium hypochlorite, vital pulp therapy

HIGHLIGHTS

- For the outcome of vital pulp therapy procedure with ProRoot® MTA, irrigation with normal saline solution was not worse than that with sodium hypochlorite.
- Discolouration occurred in vital pulp therapy-treated teeth with ProRoot® MTA irrigated with either normal saline solution or sodium hypochlorite.
- When esthetic is a concern, the combination of ProRoot® MTA with either normal saline solution or sodium hypochlorite may be contraindicated.

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Address for correspondence:
Papimon Chompu-Inwai
Division of Pediatric Dentistry, Department of Orthodontics and Pediatric Dentistry, Chiang Mai University Faculty of Dentistry, Chiang Mai, Thailand
E-mail: papimonin@hotmail.com

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INTRODUCTION

By definition of the European Society of Endodontology, vital pulp therapy (VPT) includes indirect pulp capping (IPC), direct pulp capping (DPC), partial pulpotomy (PP), and coronal pulpotomy (CP) (1). Emerging evidence has shown promising results of VPT (2–4). Mineral trioxide aggregate (MTA) is currently one of the most studied and commonly used pulp dressing materials for VPT because it results in high success (2–4). Besides the type of pulp dressing material, irrigation is also an essential VPT step to eliminate debris, pulp remnants, and microorganisms, and most importantly, to promote pulp healing. However, there is a lack of well-designed trials on the most appropriate irrigant for VPT (5).

Various irrigants used in VPT include sodium hypochlorite (NaOCl), normal saline solution (1), chlorhexidine (CHX), ethylenediaminetetraacetic acid (EDTA), and even water from dental triple-syringe (5). However, NaOCl is one of the most commonly used irrigants and its use along with MTA often results in successful outcomes (2, 3, 6). NaOCl has antimicrobial property and the ability to dissolve organic substances (7), which are essential properties for root canal treatment (RCT). Conversely, a high concentration of NaOCl is considered cytotoxic and hazardous to dental pulp stem cells (DPSCs) (8), thus may theoretically cause a disturbance in pulp healing and lower the VPT outcome. Other undesirable properties of NaOCl include its unpleasant taste and odour, damage to surrounding tissues, and alteration of the properties of the remaining tooth structure (9). Additionally, undesirable tooth discolouration following the use of MTA and NaOCl has previously been reported (10, 11).

Despite the lack of antibacterial effects (12), NSS also demonstrated high VPT success when used along with MTA in previous studies (6, 13). However, NSS is less commonly used for VPT than NaOCl (5) regardless of its high biocompatibility and non-cytotoxic properties (8) , while NaOCl usage was proposed in VPT procedures because of its antimicrobial ability (1, 14). Experimental studies have examined various calcium silicate cements (CSCs) with various irrigants and also other factors with heterogeneity in materials and methods (11, 15–17). Some studies have shown discolouration of MTA with saline (16, 17). However, some *in vitro* studies showed that immersion of MTA in NSS showed no (11) or lower discolouration (15), compared to other irrigants, including NaOCl. From the properties of NSS mentioned above, NSS may serve as the suitable irrigant for VPT.

Previous results from an *in vivo* study demonstrated that the histomorphologic response of healthy teeth which had undergone intentional DPC using calcium hydroxide ($Ca(OH)_2$) with NSS as a haemostatic agent was better than the ones which used NaOCl (18). On the other hand, a clinical trial had shown comparable cumulative success of PP using MTA, irrigated with either 2.5% NaOCl or NSS, in immature permanent molars (6). Nonetheless, tooth discolouration, following the use of ProRoot® MTA (Dentsply, Tulsa, OK, US) with different irrigants, have never been clinically investigated. Therefore, this randomised controlled clinical trial aimed to compare the treatment success and discolouration following various VPT procedures using white ProRoot® MTA, irrigated with either 2.5% NaOCl or NSS, in permanent posterior teeth of 6 to 18 years old patients.

MATERIALS AND METHODS

Study Design

This study was designed as a double-blinded, non-inferiority randomised controlled trial with 1:1 allocation ratio. The patients and the evaluators for radiographic outcomes were blinded from the type of irrigant used. However, for practical reasons and feasibility, evaluators for clinical and discolouration outcomes were not blinded. The study protocol was designed in accordance with the Declaration of Helsinki and was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand (No.51/2018) and registered in the Thai Clinical Trials Registry (TCTR20181121001). Moreover, the patient's personal information was kept confidential and unidentifiable throughout the study. The CONSORT guidelines (19) were followed when reporting this study.

Participants

Participants were recruited from 6- to 18-year-old American Society of Anesthesiologists Physical Status Classification I or II patients attending the Paediatric Dentistry Clinic, Faculty of Dentistry, Chiang Mai University. The teeth were treated with VPT between March 2018 and December 2019 and followed up for 12 months.

Ethics and Consent to Participate

Assent and informed consent forms were signed by both the patients and their legal guardians after they agreed to participate in the study.

Inclusion Criteria

Inclusion criteria were permanent posterior teeth that had: 1) caries depth ≥¾ of dentine thickness on bitewing radiograph; 2) no radiolucency at furcation, internal or external root resorption, calcification or pulp canal obliteration on periapical radiograph (teeth with minor apical changes such as widening periodontal ligament space and condensing osteitis were included (2, 3); 3) pre-operative clinical diagnosis of normal pulp, reversible pulpitis, or irreversible pulpitis by history taking which includes quality, intensity, and duration of pain and stimuli which caused pain along with recording of quality and duration of sensitivity or pain from cold testing with Endo-frost (Coltene/Whaledent, Cuyahoga Falls, OH, US); 4) no clinical signs of necrosis, swelling, pus exudate, fistula, or abnormal mobility; 5) sufficient tooth structure for restoration by resin composite (with or without orthodontic band) or stainless steel crown (SSC); 6) absence or presence of pulp exposure evaluated clinically by the operator after complete removal of caries; and 7) in cases with pulp exposure, after removal of infected and inflamed pulp tissue when indicated, there was a resilient pulp texture and bright red blood with a continuous flowing from the exposure site, which can be stopped within 10 minutes (20).

Exclusion Criteria

After following the protocol of this study (described below), teeth were excluded if they had: 1) dark or pale appearance

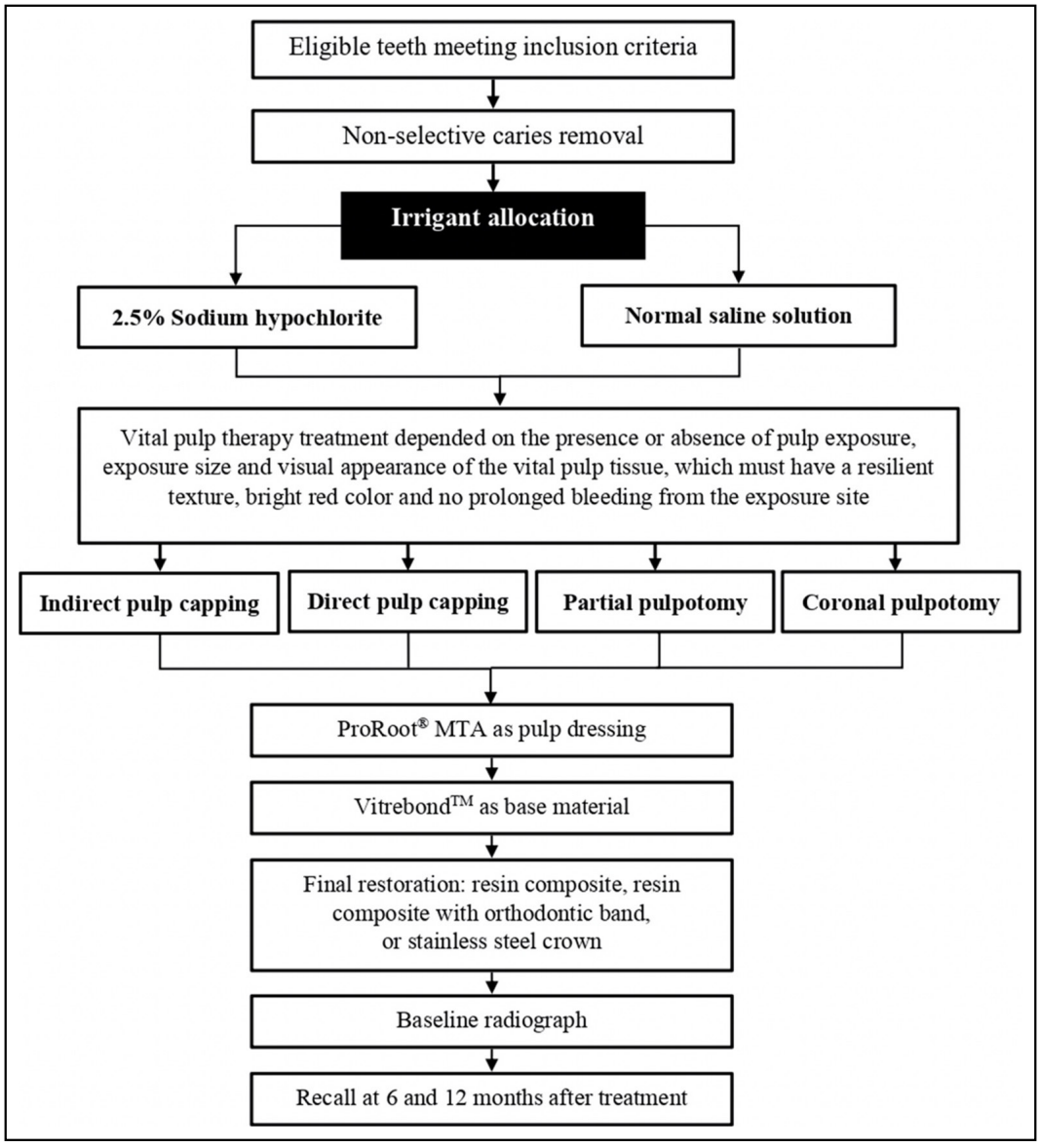

Figure 1. Flowchart of study intervention

MTA: Mineral trioxide aggregate

of pulp tissue with absence of bleeding suggesting pulp necrosis and 2) profuse, dark or prolonged bleeding that needed >10 minutes for hemostasis.

Randomisation

If the tooth met the inclusion criteria and was included in the study, it was randomly assigned to 2.5% NaOCl or NSS group. The simple randomisation method was performed using the web-generated sequence table (21) created by a postgraduate student unrelated to the study. Nonetheless, if the participant had more than 1 tooth requiring treatment, only the first 2 treated teeth were included. The first tooth (with more severe diagnosis) was randomised to one irrigant, while the second tooth would receive treatment with the other irrigant.

Treatment Protocol

Seven unblinded postgraduate students performed treatment under supervision of three calibrated instructors following the same strict protocol. Topical anesthesia (One Touch; Hager Worldwide, Concord, Ontario, Canada) was applied at the injection site before administering 4% articaine with 1:100,000 epinephrine (Septanest SP; Septodont, Saint-Maur-des-Fossés, France), then rubber dam isolation was placed. Cavity preparation was done by a high-speed round diamond bur 1–1.5 mm in diameter, and non-selective caries removal was performed by using a slow-speed round steel bur 2 mm in diameter and spoon excavation. The type of irrigant was blinded to the patient and allocated as mentioned earlier. During treatment, the surrounding dentine and pulp tissue was irrigated when needed with a total of 10–20 ml of the allocated irrigant. Haemostasis was performed by using moist cotton pellets soaked with the same irrigant. The bleeding was evaluated every 2 minutes and the time used to achieve haemostasis was recorded.

The treatment was rendered as shown in Figure 1: a) teeth with no pulp exposure would be treated with IPC and b) if pulp exposure occurred, the tooth was categorised into each treatment group, depending on the exposure size measured

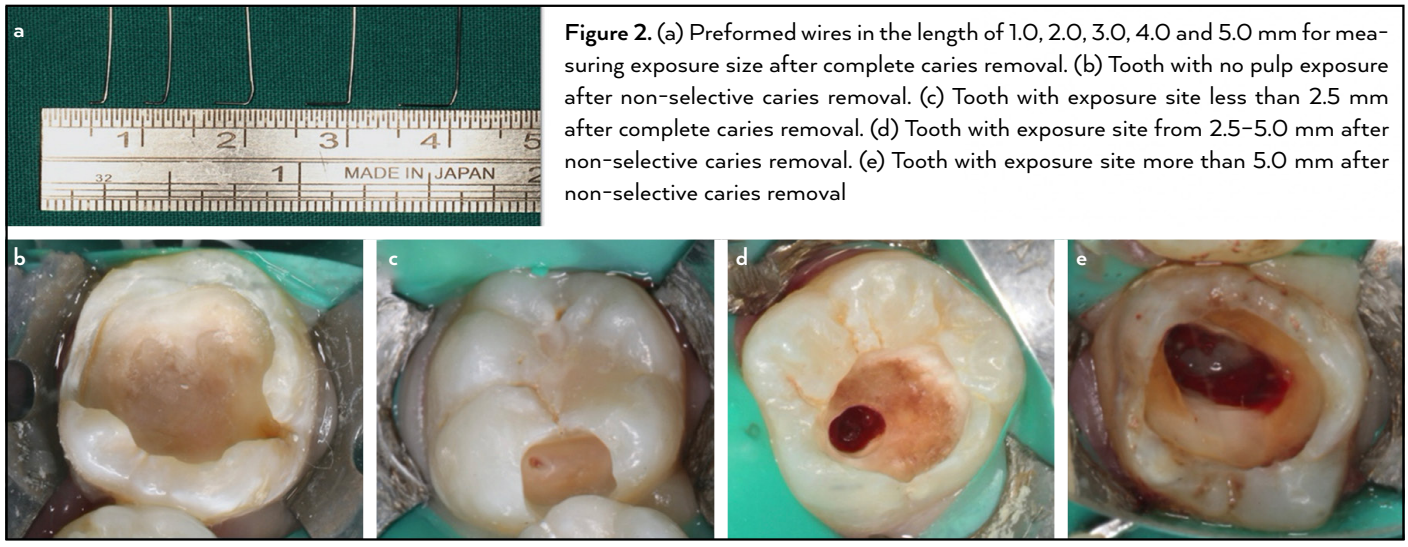

Figure 2. (a) Preformed wires in the length of 1.0, 2.0, 3.0, 4.0 and 5.0 mm for measuring exposure size after complete caries removal. (b) Tooth with no pulp exposure after non-selective caries removal. (c) Tooth with exposure site less than 2.5 mm after complete caries removal. (d) Tooth with exposure site from 2.5–5.0 mm after non-selective caries removal. (e) Tooth with exposure site more than 5.0 mm after non-selective caries removal

by preformed wires as shown in Figure 2, visual appearance, bleeding colour, and haemostasis time. DPC was carried out in teeth with exposure sizes <2.5 mm and bright red bleeding that can be stopped within 10 minutes (2, 20). PP was performed when exposures were from 2.5 to 5 mm. or when DPC could not be performed due to profuse, dark or prolonged bleeding. Pulp tissue removal was performed 2–3 mm underneath the exposure site or until healthy pulp tissue was met, by using a sterile highspeed round carbide bur 1.5 mm in diameter (3). Lastly, if the exposure size was >5 mm (22, 23) or profuse, dark or prolonged bleeding or infected pulp tissue was still present after PP, the tooth was treated by CP (23), by removing the entire coronal pulpal tissue to the orifices using a sterile highspeed round carbide bur and spoon excavator. White ProRoot® MTA was mixed following the manufacturer's instructions and placed as a pulp dressing material in both groups with a thickness of 1.5–3 mm. A layer of resin modified glass ionomer (Vitrebond™; 3M™ ESPE™, St Paul, MN, US) was applied over MTA. Then, restoration of the tooth was performed in the same visit by either resin composite (FiltekTM Z350 XT; 3M™ ESPE™) with or without orthodontic band or SSC (3M™ ESPE™). An immediate post-operative periapical radiograph, using paralleling technique, was obtained to serve as a baseline. Baseline variables, including sex, age, tooth type, tooth arch location, diagnosis, periapical status, hemostasis time (in cases with pulp exposure), type of VPT, and final restoration, were recorded. Patients were called back at 6 and 12 months after treatment for clinical, radiographic, and discolouration evaluations.

Evaluation of the Outcomes

In this study, the primary outcome was the success of VPT; the tooth required both clinical and radiographic success to be considered as success. The secondary outcome was discolouration; percentages of discolourations between both groups were compared.

Clinical and discolouration evaluations were conducted by unblinded postgraduate and undergraduate dental students under the supervision of the same three instructors who supervised the treatment. Criteria for clinical failure were presence of signs or symptoms of reversible or irreversible pulpitis, pain on percussion, swelling, pus exudates, fistulae in soft and periodontal tissues, or abnormal tooth mobility. Apart from the VPT success and failure, perceptible grey discolouration was also clinically evaluated. Because SSC and orthodontic band could mask the discolouration, only teeth restored with resin composite were included for discolouration evaluation. Perceptible grey discolouration of the tooth was recorded as "presence" or "absence" at each follow-up visit.

Radiographic evaluations were performed by two blinded postgraduate students who did not participate in the study and were calibrated with a paediatric dentist who has more than 10 years of VPT experience and was one of the instructors who supervised the treatment (gold standard). After calibration of 24 sets of radiographs, the percentage of agreement between the gold standard and the two examiners were 91.6% and 100%. Inter-examiner reliability for radiographic evaluation was 91.6%. Re-evaluation was performed 1 month later, intra-examiner reliabilities for radiographic evaluation were 95.8% and 100%. Radiographs were independently evaluated by both evaluators. Follow-up radiographs were compared to the baseline. Criteria for radiographic failure were loss of lamina dura, discontinued root formation, or more advanced periapical lesion. If the treated tooth had any clinical or radiographic failure criteria, it was determined as failure.

Statistical Analysis

The hypothesis of this study was that the MTA-VPT success in teeth irrigated with NSS was not worse than those with NaOCl, by a pre-specified margin of -5% (NSS– NaOCl>-5%). The pre-specified margin was established according to the study by Özgür et al. (6), which reported 94.4% and 100% cumulative success rates of PP with NaOCl and NSS, respectively. If the lower margin of the 95% CI absolute difference of MTA-VPT success between two groups is >-5%, NSS is considered non-inferior to NaOCl. However, if the lower margin is less than -5%, the non-inferiority hypothesis between the VPT success of both groups could not be proved. Sample size calculation was done by using an automatic program of binary outcome for non-inferiority trial (24) with 5% non-inferiority limit, 90%

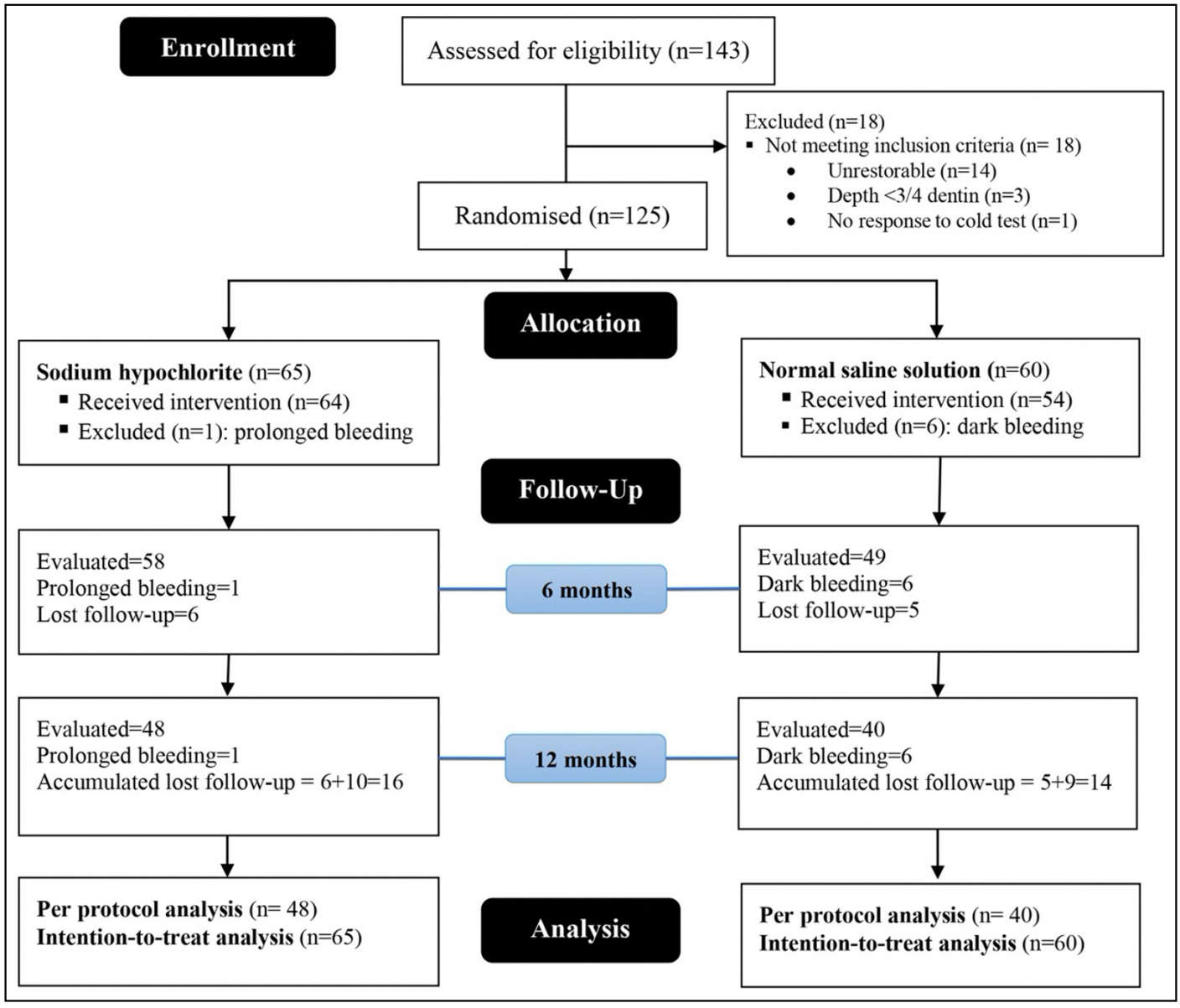

Figure 3. Flowchart of participants in the study

precision of test and 5% type I error, 40 samples were required in each group. To compensate for follow-up lost, an approximately additional 25% of the sample size was added. Therefore, at least 50 teeth were required in each group.

Data analysis was generated by SPSS 25.0 (SPSS Science, Chicago, IL, US) and STATA 16.0 (Stata Corp, College Station, TX, US) with the level of significant difference at $p<0.05$. The baseline variables in both groups were compared for the different population analyses, including per protocol analyses and intention-to-treat analyses at 12 months.

The per protocol (complete case) analysis was conducted to compare VPT success between both irrigants. However, because of missing data due to lost follow-up cases, the intention-to-treat (ITT) analyses, including analyses assuming that all lost cases had success or failures, the best-, and worst-case scenarios were also conducted to simulate the outcome if all participants were evaluated at the final follow-up time point. The 'best-case' scenario is that all participants with missing outcomes in the NSS group had success, and all those with missing outcomes in the NaOCl group had failures; the 'worst-case' scenario is the converse. Absolute difference with 95% confidence interval (CI) was used for reporting the percentage differences of VPT outcomes and discolourations between the NaOCl and NSS groups. Kaplan–Meier survival probabilities were also calculated and compared using the log rank test.

RESULTS

Figure 3 shows the flowchart of participants in the study. A total of 143 teeth were assessed for eligibility with 125 teeth meeting the inclusion criteria and were initially included and randomised to each irrigant, 65 in the NaOCl and 60 in the NSS groups. Ten patients had two treated teeth, one for each group. The recall rates, including all cases that were randomised, were 76% (95/125) at the 12-month follow-up. Therefore, the population for a per protocol analysis at 12 months was 88 (48 in

TABLE 1. Baseline variables of the sodium hypochlorite and normal saline solution groups for the different

	Population			
	Intention-to-treat analysis		Per protocol analysis	
	NaOCl	NSS	NaOCl	NSS
Number of teeth, n	65	60	48	40
Sex, % (n/N)				
Male	40.0 (26/65)	45.0 (27/60)	41.7 (20/48)	45.0 (18/40)
Female	60.0 (39/65)	55.0 (33/60)	58.3 (28/48)	55.0 (22/40)
Age, (years)				
Mean±SD	10.4±2.0	10.2±1.9	10.6±2.2	10.3±2.0
Range	6.5–16.3	7.1–14.0	6.5–16.3	7.1–14.0
Tooth type, % (n/N)				
Molar	98.5 (64/65)	96.7 (58/60)	97.9 (47/48)	97.5 (39/40)
Premolar	1.5 (1/65)	3.3 (2/60)	2.1 (1/48)	2.5 (1/40)
Tooth arch location, % (n/N)				
Maxillary	46.2 (30/65)	43.3 (26/60)	39.6 (19/48)	37.5 (15/40)
Mandibular	53.8 (35/65)	56.7 (34/60)	60.4 (29/48)	62.5 (25/40)
Diagnosis, % (n/N)				
Normal pulp	12.3 (8/65)	16.7 (10/60)	14.6 (7/48)	15.0 (6/40)
Reversible pulpitis	46.2 (30/65)	45.0 (27/60)	47.9 (23/48)	42.5 (17/40)
Irreversible pulpitis	41.5 (27/65)	38.3 (23/60)	37.5 (18/48)	42.5 (17/40)
Periapical status, % (n/N)				
Normal	63.1 (41/65)	73.3 (44/60)	64.6 (31/48)	70.0 (28/40)
Early periapical lesions	36.9 (24/65)	26.7 (16/60)	35.4 (17/48)	30.0 (20/40)
Type of VPT, % (n/N)				
Indirect pulp capping	26.2 (17/65)	31.7 (19/60)	29.2 (14/48)	32.5 (13/40)
Direct pulp capping	20.0 (13/65)	18.3 (11/60)	20.8 (10/48)	22.5 (9/40)
Partial pulpotomy	29.2 (19/65)	23.3 (14/60)	27.1 (13/48)	17.5 (7/40)
Coronal pulpotomy	24.6 (16/65)	26.7 (16/60)	22.9 (11/48)	27.5 (11/40)
Haemostasis time in cases with pulp exposure, (minutes)				
Mean±SD	3.0±2.2	2.7±1.3	2.7±1.2	2.8±1.5
Range	2–15	2–8	2–6	2–8
Final restoration, % (n/N)				
Resin composite				
Single surface	23.1 (15/65)	21.7 (13/60)	18.8 (9/48)	20 (8/40)
Multiple surfaces	43.1 (28/65)	36.7 (22/60)	37.5 (18/48)	37.5 (15/40)
Stainless steel crown	33.8 (22/65)	41.7 (25/60)	43.7 (21/48)	42.5(17/40)

NaOCl: Sodium hypochlorite, NSS: Normal saline solution, n: Number of teeth with mentioned variable in each population, N: All included teeth in each population, SD: Standard deviation, VPT: Vital pulp therapy

the NaOCl, 40 in the NSS groups). The population for an intention-to-treat analysis was 125 (65 in the NaOCl, 60 in the NSS groups). Table 1 displays the baseline variables between two groups for the different population analyses.

Table 2 shows absolute differences of VPT success between NSS and NaOCl groups at 12 months after treatment. Using a per protocol analysis, NSS had 2.08% (95% CI -1.95, 6.1) higher success than NaOCl at 12 months. Because the lower margin of the absolute differences of VPT success were >-5%, NSS appears to be non-inferior to NaOCl for VPT success. An ITT analysis was also performed and shown in Table 2. When all lost cases were assumed to have success, the absolute difference was -1.5 (-4.45, 1.45), confirming that NSS was not worse than NaOCl for VPT success. When all lost cases were assumed to have failures, the difference was inconclusive. For the worst-case scenario, NSS was worse than NaOCl. On the other hand, for the best-cases scenario, NSS was superior to NaOCl.

Until the end of this study, there was only one failure in the NaOCl group that occurred at 1 month after the tooth was treated with PP. The patient exhibited painful pulpitis and was referred for RCT. The failed tooth was initially diagnosed with irreversible pulpitis with minor periapical changes, widening periodontal ligament space. The survival probability of teeth irrigated with NaOCl was 0.98 (95% CI: 0.88–1.00) and there was no failure in the NSS group (survival probability=1). There was no significant difference in the survival probabilities between the studied groups (log rank test=0.93). Figure 4 demonstrates examples of radiographic success of teeth after MTA-VPT with both irrigants.

Among the 107 teeth that received VPT, 57 teeth were restored with resin composite without orthodontic band, overall discolouration was 71.9% (41/57), 80% (24/30) and 63% (17/27) in the NaOCl and NSS groups. Although NSS displayed less percentage of discolouration, the difference between percentages

TABLE 2. Success of vital pulp therapy using mineral trioxide aggregate, with either sodium hypochlorite or normal saline solution

Analysis	Intervention group		Absolute difference NSS-NaOCl (95% CI)	Interpretation
	NaOCl % (n/N) success	NSS % (n/N) success		
Per protocol analysis (complete case)	97.9 (47/48)	100 (40/40)	2.08 (-1.95, 6.1)	NSS is non-inferior to NaOCl
Intention-to-treat analysis				
All lost cases had success	98.5 (64/65)	100 (60/60)	-1.5 (-4.45, 1.45)	NSS is non-inferior to NaOCl
All lost cases had failures	72.3 (47/65)	71.7 (43/60)	0.6 (-15.2, 16.3)	Inconclusive results
Worst-case scenario	98.5 (64/65)	71.7 (43/60)	-26.8 (138.6, -15.0)	NSS is worse than NaOCl
Best-case scenario	72.3 (47/65)	100 (60/60)	27.7 (16.8, 38.6)	NSS is superior to NaOCl

NSS: Normal saline solution, NaOCl: Sodium hypochlorite, n: Number of teeth with success of vital pulp therapy in each intervention group, N: All included teeth in each intervention group, CI: Confidence interval

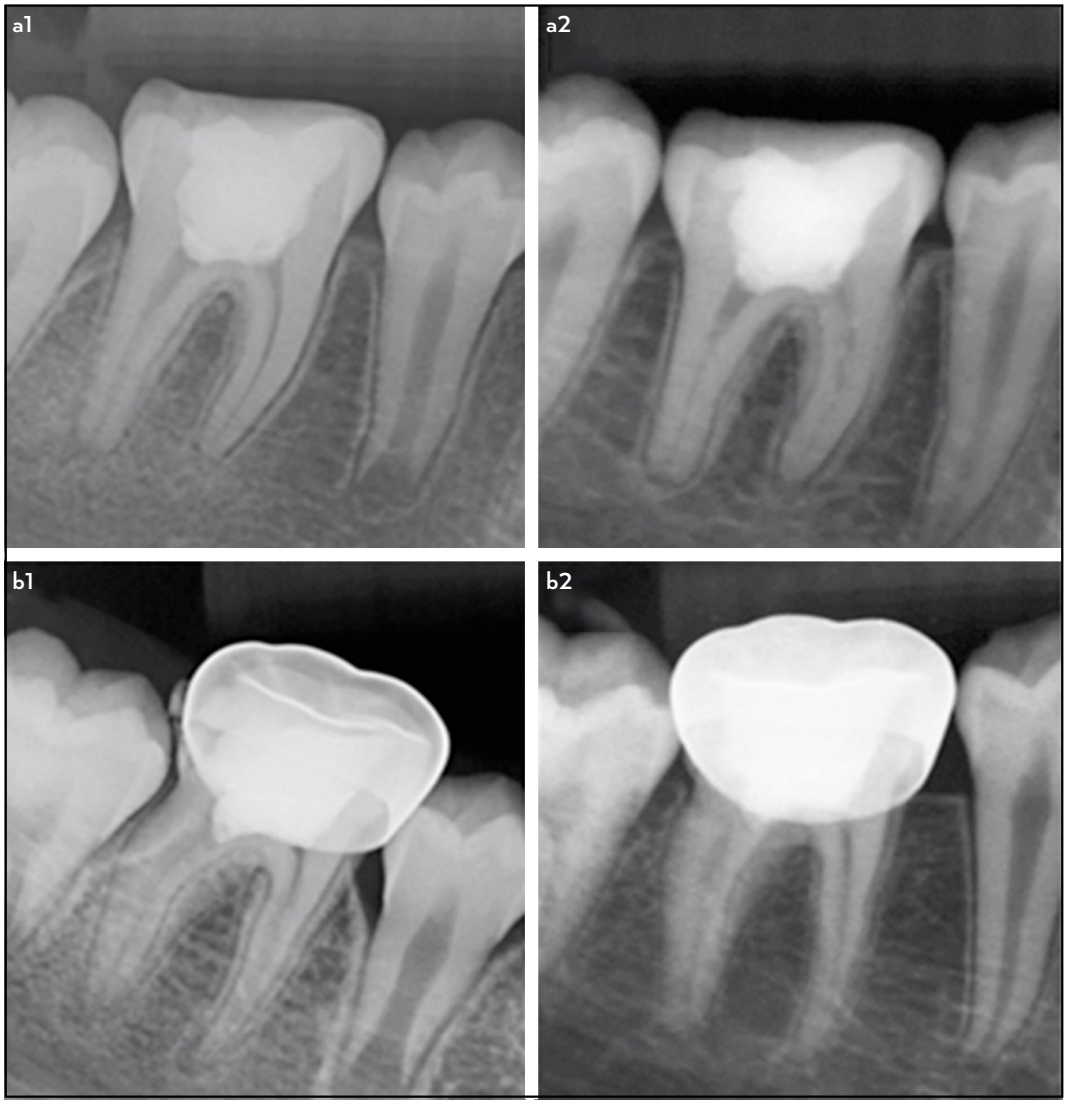

Figure 4. (a) Permanent right mandibular first molar treated by White ProRoot® MTA coronal pulpotomy using sodium hypochlorite as irrigant (a1) Immediate postoperative, (a2) improvement of periapical status is noticeable at follow-up. (b) Permanent right mandibular first molar treated by White ProRoot® MTA coronal pulpotomy using normal saline as irrigant (b1) Immediate postoperative: the cement remnant on distal surface was removed after radiograph taken, (b2) improvement of periapical status on the mesial and distal roots is noticeable at follow-up

MTA: Mineral trioxide aggregate

TABLE 3. Perceptible grey discolouration percentage by treatment procedures

	Total* N	Perceptible grey discolouration	
		Yes	No
Type of vital pulp therapy procedures, n/N (%)			
Indirect pulp capping	15/57 (26.3)	6/15 (40.0)	9/15 (60.0)
Direct pulp capping	15/57 (26.3)	13/15 (86.7)	2/15 (13.3)
Partial pulpotomy	18/57 (31.6)	16/18 (88.9)	2/18 (11.1)
Coronal pulpotomy	9/57 (15.8)	6/9 (66.7)	3/9 (33.3)

*: Only VPT-treated teeth restored with direct resin composite restorations were included for analysis (Total N=57). n: Number of teeth with discolouration in each type of vital pulp therapy procedure, N: All included teeth in each type of vital pulp therapy procedure

of discolouration by both irrigants was inconclusive (difference -17%; 95% CI: -40.0- 6.2; p=0.15). Perceptible grey discolouration percentage by treatment procedures is shown in Table 3. Figure 5 shows discolouration of teeth irrigated by both irrigants.

DISCUSSION

Although MTA has been one of the most studied and commonly used pulp dressing materials for VPT (4, 25), the evidence regarding the most suitable irrigant that should accompany its use is lacking. Irrigants used in endodontics mostly derive from studies based on RCT which is a maximally invasive surgically-based treatment that aims to effectively clean the complicated root canal system (7). On the other hand, VPT is a minimally invasive biologically-based treatment that aims to preserve pulp vitality. The difference in treatment objectives may influence the choice of irrigant between these two procedures. The result of this study will hopefully shed light on the most suitable irrigant, when used in combination with ProRoot® MTA, that can maximise the outcome of VPT while minimising the undesirable tooth discolouration.

In a per protocol analysis, the MTA-VPT success in the NSS group appears to be not worse than that in the NaOCl group at 12 months, regardless of the broad spectrum of diagnosis or type of VPT procedures performed. Moreover, the difference of survival probabilities between MTA-VPT, irrigated with NSS and with NaOCl could not be observed. Few previous trials using NSS in teeth with irreversible pulpitis along with different calcium silicate-based cements (CSCs), including MTA, demonstrated success between 73–95% at 1 year (13, 26). Antibacterial effect, biocompatibility, and bioactive properties are major desirable properties of materials which would promote successful VPT outcomes (27). CSCs have excellent biocompatibility, together with antibacterial and bioactive properties (4) and its prolonged contact with the pulp tissue may make the antibacterial effect shortly provided by irrigant less important. Therefore, the lack of antibacterial effect of NSS may not be a concern when it is used in combination with CSCs, following the correct evaluation of pulp status and sufficient removal of infected and inflamed pulp tissue.

Missing outcome data continues to be a common problem in randomised controlled trials; it can reduce the power and efficiency of a study and, unfortunately, can also lead to biased results. The ITT principle, in short, "analyse as randomised", is recognised as an important protection against bias by preserving the benefits of randomisation–namely balancing both known and unknown factors and eliminating selection bias (28). Despite using additional ITT analyses to investigate the effects of missing data on the outcome of this study, the drastic difference in the best-case and worse-case scenario were too extreme, with a tremendous contradiction in results from a similar study by Özgür et al. (6), which reported 94.4% and 100% two-year cumulative success rates of PP with NaOCl and NSS, respectively. Consequently, if all lost cases were assumed to have success, similar to the high success rates found in the previously mentioned study, NSS was confirmed to be not worse than NaOCl.

The only failed case in this study, which had a diagnosis of irreversible pulpitis and was treated by PP using NaOCl, occurred within the first month after treatment. Although earlier studies have shown success in treating irreversible pulpitis by PP, the procedure was carried out according to subjective clinical features such as pulpal appearance, bleeding color, and hemostasis time (3, 6). In this case, the failure may be caused by insufficient debridement or extensive infection beyond the coronal portion, suggesting that a more invasive treatment like CP or RCT should have been carried out as adequate management of the infected and inflamed pulp tissue is essential for treatment success.

The balance between the irrigant's role in disinfection and cytotoxicity may be essential in determining the appropriate choice of irrigant for VPT. Although NaOCl is the most common irrigant used among endodontists (7), its right concentration for VPT is unknown. The concentration range between 0.5–5.25% NaOCl was clinically used in other VPT studies (5) and the concentration of 2.5% was chosen for this present study as it was the frequently used concentration (2, 3, 6, 22, 23). These clinically used concentrations were considered very high compared to the concentrations previously reported to be cytotoxic in *in vitro* studies (29, 30). A study found that there was no significant difference in human pulp cell proliferation after exposure to 0.08% NaOCl compared to NSS, but a significant decline was found after the concentration increased to 0.16% and cell death appeared at 0.33% (29). Additionally, NSS showed significantly higher DPSCs attachment and survival compared to those with 5.25% NaOCl in *in vitro* (8) and better histomorphologic after $Ca(OH)_2$ DPC compared to those with 2.5% NaOCl in *in vivo* (18). The non-cytotoxicity and excellent biocompatibility properties of NSS (8) may benefit pulp healing after VPT. Apparently, there was no clinical difference of VPT

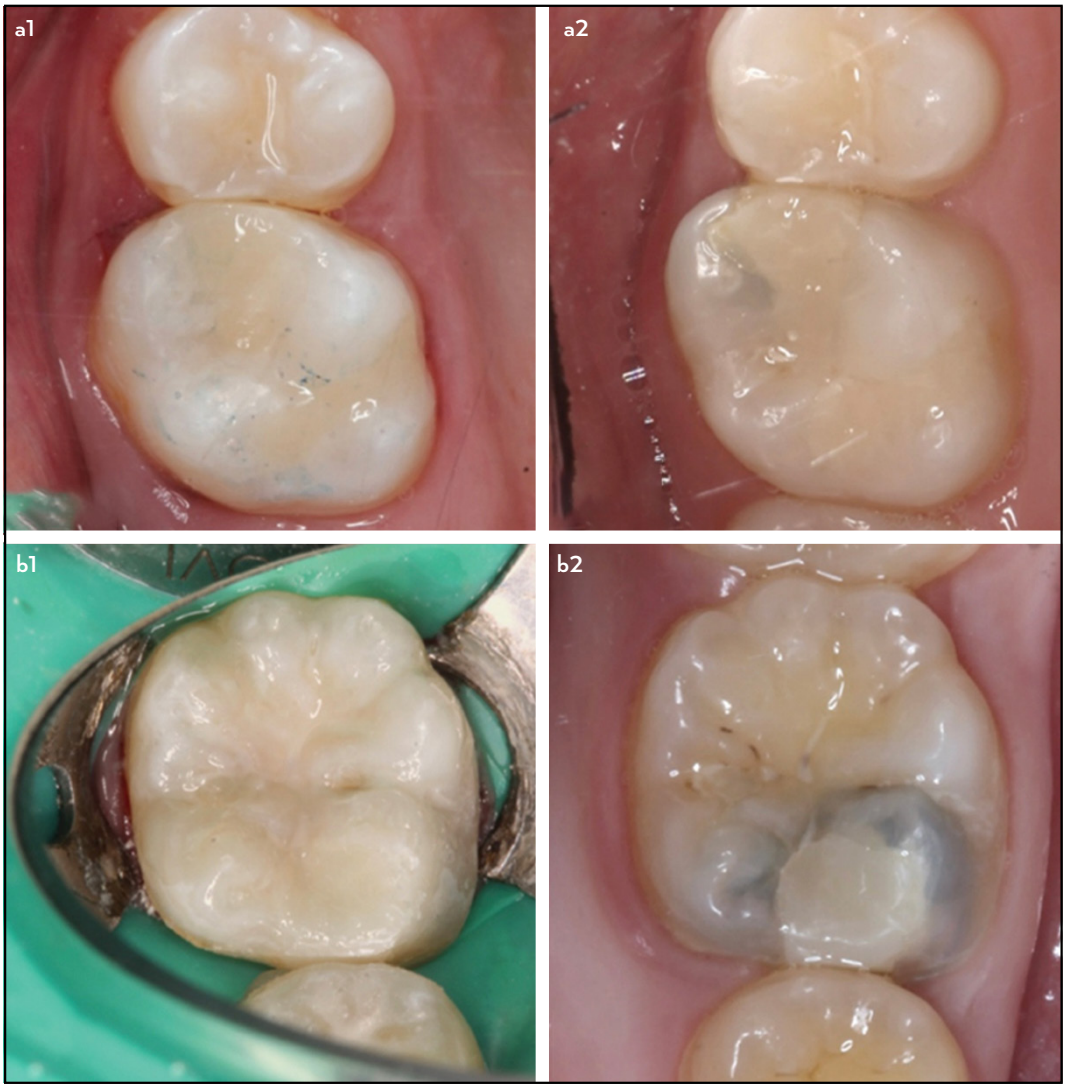

Figure 5. (a) Permanent right maxillary first molar treated by White ProRoot® MTA direct pulp capping using sodium hypochlorite as irrigant (a1) Immediate postoperative, (a2) grey discolouration is noticeable at follow-up. (b) Permanent left mandibular first molar treated by White ProRoot® MTA partial pulpotomy using normal saline as irrigant (b1) Immediate postoperative, (b2) grey discolouration isnoticeable at follow-up

MTA: Mineral trioxide aggregate

outcome between using NaOCl and NSS in this study. Histomorphological results from *in vivo* studies showed that NaOCl did not affect pulp repair of healthy teeth treated by intentional $Ca(OH)_2$ DPC which used NaOCl as a hemostatic agent for 20 seconds and washed with NSS afterwards (31) and dentin bridge formation still occurred even when only NSS was used as a hemostatic agent with both $Ca(OH)_2$ (32, 33) and MTA (33) as the pulp capping material. Correspondingly, a recent clinical study found that 2.5% NaOCl irrigation in cariously exposed teeth treated with DPC had a significantly lower early painful failures compared to using NSS (p=0.0008) (34). The outcome was assumably caused by NaOCl's ability to dissolve necrotic pulp tissues. This property of NaOCl can be valuable to VPT procedures such as DPC, which does not include mechanical debridement during the procedure. Further studies are recommended regarding the effect of NaOCl cytotoxicity and its significance on clinical outcomes of VPT. Moreover, compared to NSS, 3% and 5% NaOCl significantly decreased dentin elastic modulus and flexural strength (9) while its effect on bond strength remains controversial (35). Therefore, adverse effects of irrigants on other aspects such as the remaining tooth structure or bonding of restoration should not be neglected as adequate coronal seal is necessary for successful outcomes (36).

Perceptible grey discolourations were found in both groups in this study, contradicting a previous *in vitro* study demonstrating unnoticeable grey discolouration by visualisation when NSS was used with ProRoot® MTA (11). However, the difference in percentages of discoloured teeth caused by these two irrigants must be interpreted with caution because the wide 95% CI indicates that the difference remains inconclusive. Unavoidably, a high number of teeth, 42 with SSC and 8 with orthodontic band, were excluded from discolouration evaluation. Nevertheless, blood contamination may be the reason of these high discolourations (67–89%) in the VPT procedures with pulp exposure. Previous *ex vivo* studies demonstrated that blood

contamination, which is considered as another factor for discolouration, potentiated tooth discolouration after application of various CSCs (16). This result is also consistent with clinical studies reporting discolouration after DPC (2) and PP (3), using MTA with NaOCl. Furthermore, although NSS was used with MTA, discolouration of pulpotomised immature traumatised anterior teeth was also previously reported (37). Surprisingly, despite its larger exposure size, discolouration in the CP subgroup was less than in the DPC and PP subgroup. This result may be caused by the more invasive and destructive procedure of CP, leading to a higher number of cases requiring SSC restorations, thus excluding them from discolouration evaluation. Another remarked observation is the 40% discolouration in the IPC treated teeth, despite the absence of blood contamination in this group. This result can be the effect of light irradiation and anerobic conditions, which were demonstrated to influence discolouration of CSCs in a previous *in vitro* study (38). Therefore, preventing or reducing discolouration after MTA-VPT treatment could not be done by controlling only one factor such as the irrigant used, owing to the many factors that can influence discolouration as mentioned above.

Although white MTA was developed to encounter discolouration caused by the original grey MTA (4), the high overall perceptible grey discolouration from this study showed that it did not achieve this purpose. An oxidative reaction between NaOCl and bismuth oxide, the radiopacifier in MTA, was proposed to be the cause of this adverse event (10). Moreover, earlier studies found that the presence of blood contamination was another factor which accentuated discolouration (16, 17). Despite the high success rates in both irrigant groups, NSS use with MTA, instead of NaOCl, could not eliminate this discolouration problem observed clinically despite unnoticeable discolouration shown in *in vitro* study (11). Although grey discolouration after treatment may not be a major concern as only posterior teeth were included in this study, it would most likely cause a more negative effect on patients that require VPT in esthetic concerned areas. Therefore, the use of other CSCs products which reported less discolouration may be a better option to reduce this adverse effect. Consequently, clinical studies aimed to assess color stability of other claimed non-staining CSCs with different irrigants should be encouraged to determine optimal materials and irrigants for VPT procedure.

The limitations of this study should not be overlooked. First, the degree of discolouration between groups could not be compared as the outcome was obtained by visualisation of dental professionals and reported in a dichotomous manner (absence or presence). Future studies, using devices or other methods which can provide quantitative and more objective results, are suggested. Second, several subjects were excluded from discolouration evaluation because SSC or resin composite with orthodontic band were placed as their final restorations, which led to an inconclusive result of difference in percentages of discoloured teeth between NaOCl and NSS. Obtaining a larger sample size in future studies may elicit more accurate results regarding discolouration after VPT treatment.

CONCLUSION

From the result of this study, in terms of VPT outcome, NSS had the same efficacy as NaOCl and can be considered as an alternative irrigant for VPT with ProRoot® MTA in young permanent teeth because of its biocompatibility which can facilitate safe irrigation and prevent hazards from NaOCl spillage in partially erupted or heavily compromised teeth which may encounter difficulty to isolate. However, both NSS and NaOCl caused perceptible grey discolouration. Thus, the use of other CSCs which claim to be non-staining should be more appropriate in treating teeth that require high esthetics.

Disclosures

Ethics Committee Approval: The study was approved by the Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand Human Experimentation Ethics Committee (no: 51/2018, date: 25/02/2018).

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REFERENCES

1. Duncan HF, Galler KM, Tomson PL, Simon S, El-Karim I, Kundzina R, et al. European society of endodontology position statement: Management of deep caries and the exposed pulp. Int Endod J 2019; 52(7):923–34. [CrossRef]
2. Parinyaprom N, Nirunsittirat A, Chuveera P, Na Lampang S, Srisuwan T, Sastraruji T, et al. Outcomes of direct pulp capping by using either Proroot mineral trioxide aggregate or Biodentine in permanent teeth with carious pulp exposure in 6- to 18-year-old patients: A randomized controlled trial. J Endod 2018; 44(3):341–8. [CrossRef]
3. Uesrichai N, Nirunsittirat A, Chuveera P, Srisuwan T, Sastraruji T, Chompu-inwai P. Partial pulpotomy with two bioactive cements in permanent teeth of 6- to 18-year-old patients with signs and symptoms indicative of irreversible pulpitis: A noninferiority randomized controlled trial. Int Endod J 2019; 52(6):749–59. [CrossRef]
4. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review--part iii: Clinical applications, drawbacks, and mechanism of action. J Endod 2010; 36(3):400–13. [CrossRef]
5. Munir A, Zehnder M, Rechenberg DK. Wound lavage in studies on vital pulp therapy of permanent teeth with carious exposures: A qualitative systematic review. J Clin Med 2020; 9(4):984. [CrossRef]
6. Ozgur B, Uysal S, Gungor HC. Partial pulpotomy in immature permanent molars after carious exposures using different hemorrhage control and capping materials. Pediatr Dent 2017; 39(5):364–70.
7. American Association of Endodontists. Root canal irrigants and disinfectants. Endodontics: Colleagues for Excellence. Chicago: American Association of Endodontists; 2011. p. 1–7.
8. Sutimuntanakul S SH, Lerdarksornpaiboon W, Songtrakul K. Effect of root canal irrigants on attachment and survival of dental pulp stem cells. M Dent J 2016; 36:375–92.
9. Grigoratos D, Knowles J, Ng Y-L, Gulabivala K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int Endod J 2001; 34(2):113–9. [CrossRef]
10. Camilleri J. Color stability of white mineral trioxide aggregate in contact with hypochlorite solution. J Endod 2014; 40(3):436–40. [CrossRef]
11. Metlerska J, Dammaschke T, Lipski M, Fagogeni I, Machoy-Mokrzyńska A, Nowicka A. Effect of citric acid on color changes of calcium silicate-based cements an *in vitro* study. Appl Sci 2021; 11:2339. [CrossRef]

12. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod 1994; 20(6):276–8. [CrossRef]
13. Nosrat A, Seifi A, Asgary S. Pulpotomy in caries-exposed immature permanent molars using calcium-enriched mixture cement or mineral trioxide aggregate: A randomized clinical trial. Int J Paediatr Dent 2013; 23(1):56–63. [CrossRef]
14. AAE position statement on vital pulp therapy. J Endod 2021; 47(9):1340–4. [CrossRef]
15. Sobhnamayan F, Adl A, Ghanbaran. Effect of different irrigation solutions on the colour stability of three calcium silicate-based materials. J Dent Biomater 2017;4:373–8.
16. Shokouhinejad N, Nekoofar MH, Pirmoazen S, Shamshiri AR, Dummer PM. Evaluation and comparison of occurrence of tooth discoloration after the application of various calcium silicate-based cements: An *ex vivo* study. J Endod 2016; 42(1):140–4. [CrossRef]
17. Farrugia C, Baca P, Camilleri J, Arias Moliz MT. Antimicrobial activity of proroot MTA in contact with blood. Sci Rep 2017;7:41359. [CrossRef]
18. Accorinte Mde L, Loguercio AD, Reis A, Holland R. Effects of hemostatic agents on the histomorphologic response of human dental pulp capped with calcium hydroxide. Quintessence Int 2007; 38(10):843–52.
19. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. BMJ 2010; 340:c332. [CrossRef]
20. Bogen G, Chandler NP. Pulp preservation in immature permanent teeth. Endod Topics 2010; 23(1):131–52. [CrossRef]
21. Dallal G. Randomization.Com. Avaliable at: http://www.jerrydallal.com/random/randomize.htm. Accessed March 1, 2018.
22. Chailertvanitkul P, Paphangkorakit J, Sooksantisakoonchai N, Pumas N, Pairojamornyoot W, Leela-Apiradee N, et al. Randomized control trial comparing calcium hydroxide and mineral trioxide aggregate for partial pulpotomies in cariously exposed pulps of permanent molars. Int Endod J 2014; 47(9):835–42. [CrossRef]
23. Ngamthuam I, Manmontri C, Chompu-inwai P, Phinyo P, Nirunsittirat A, Chaipattanawan N. Outcomes of coronal pulpotomy on permanent first molars in children: A retrospective cohort study. Clin Oral Investig 2023; 27:1–16. [CrossRef]
24. Sealed Envelope Ltd. Power calculator for binary outcome non-inferiority trial. Avaliable at: https://www.sealedenvelope.com/power/binary-non-inferior/. Accessed March 1, 2018.
25. Parirokh M, Torabinejad M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: An updated overview - part i: Vital pulp therapy. Int Endod J 2018; 51(2):177–205. [CrossRef]
26. Asgary S, Eghbal MJ. Treatment outcomes of pulpotomy in permanent molars with irreversible pulpitis using biomaterials: A multi-center randomized controlled trial. Acta Odontol Scand 2013; 71(1):130–6. [CrossRef]
27. Wang Z. Bioceramic materials in endodontics. Endod Topics 2015; 32(1):3–30.
28. Bell ML, Fiero M, Horton NJ, Hsu CH. Handling missing data in RCTs; a review of the top medical journals. BMC Med Res Methodol 2014; 14:118.
29. Essner MD, Javed A, Eleazer PD. Effect of sodium hypochlorite on human pulp cells: An *in vitro* study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(5):662–6. [CrossRef]
30. Vouzara T, Koulaouzidou E, Ziouti F, Economides N. Combined and independent cytotoxicity of sodium hypochlorite, ethylenediaminetetraacetic acid and chlorhexidine. Int Endod J 2016; 49(8):764–73. [CrossRef]
31. Elias RV, Demarco FF, Tarquinio SB, Piva E. Pulp responses to the application of a self-etching adhesive in human pulps after controlling bleeding with sodium hypochlorite. Quintessence Int 2007; 38(2):e67–77.
32. Accorinte ML, Loguercio AD, Reis A, Costa CA. Response of human pulps capped with different self-etch adhesive systems. Clin Oral Investig 2008; 12(2):119–27. [CrossRef]
33. Accorinte Mde L, Holland R, Reis A, Bortoluzzi MC, Murata SS, Dezan E, Jr., et al. Evaluation of mineral trioxide aggregate and calcium hydroxide cement as pulp-capping agents in human teeth. J Endod 2008; 34(1):1–6.
34. Ballal NV, Duncan HF, Rai N, Jalan P, Zehnder M. Sodium hypochlorite reduces postoperative discomfort and painful early failure after carious exposure and direct pulp capping-initial findings of a randomized controlled trial. J Clin Med 2020; 9(8):2408. [CrossRef]
35. Abuhaimed T, Abou Neel E. Sodium hypochlorite irrigation and its effect on bond strength to dentin. Biomed Res Int 2017; 2017:1930360. [CrossRef]
36. Akhlaghi N, Khademi A. Outcomes of vital pulp therapy in permanent teeth with different medicaments based on review of the literature. Dent Res J (Isfahan) 2015; 12(5):406–17. [CrossRef]
37. Abuelniel G, Duggal M, Kabel N. A comparison of mta and biodentine as medicaments for pulpotomy in traumatized anterior immature permanent teeth: A randomized clinical trial. Dent Traumatol 2020;36:400–10.
38. Vallés M, Mercadé M, Duran-Sindreu F, Bourdelande JL, Roig M. Influence of light and oxygen on the color stability of five calcium silicate-based materials. J Endod 2013; 39(4):525–8. [CrossRef]