ORIGINAL ARTICLE



Comparing the incidence of postoperative pain after root canal filling with warm vertical obturation with resin-based sealer and sealer-based obturation with calcium silicate-based sealer: a prospective clinical trial

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Received: 25 September 2020 / Accepted: 27 January 2021 / Published online: 8 February 2021 © The Author(s), under exclusive licence to Springer-Verlag GmbH, DE part of Springer Nature 2021

Abstract

Objective This prospective clinical study compares postoperative pain after single-visit, non-surgical root canal treatment of teeth with irreversible pulpitis using two different root canal filling techniques.

Material and methods All cases were treated by endodontic residents with a standardized protocol (minimum apical size 35) and filled with one of the two techniques: warm vertical compaction technique (WVT) with gutta percha and epoxy resin-based sealer (AH Plus Jet Root Canal Sealer, Dentsply Maillefer, York, PA, USA) or sealer-based filling technique (SBT) with single cone gutta percha and calcium silicate-based sealer (EndoSequence BC Sealer, Brasseler, Savannah, GA, USA). Surveys were given to participating patients to record pain intensity on a numeric rating scale (NRS, 0–10) at 4, 24, and 48 h postoperatively. Statistical significance was set at 0.05 level.

Results One hundred ninety-four surveys were distributed over eighteen months. Ninety-two patients returned the survey (41 WVT and 51 SBT), of which 38% were asymptomatic irreversible pulpitis cases. The NRS values reduced over time for both techniques. No statistical difference was found between the two groups at the three time points assessed (p > 0.05). Postoperative pain was related to age, gender, presence of preoperative pain, and sealer extrusion (p < 0.05), however not related to preoperative periapical symptoms (percussion/palpation), dental arch, root type, and experience of the provider (p > 0.05).

Conclusions The intensity of postoperative pain for the two obturation techniques was equivalent at evaluated time points. **Clinical relevance** The obturation technique does not influence postoperative pain. After endodontic treatment of symptomatic irreversible pulpitis teeth, the pain subsides in 48 h regardless of the technique. **Trial registration** ClinicalTrials.gov ID: NCT04462731

Keywords Bioceramic sealer · Calcium silicate-based sealer · Epoxy resin-based sealer · Postoperative pain · Root canal filling

Introduction

The incidence of postoperative pain after non-surgical root canal treatment is reported to be in the range of 0 to 48% [1–3]. The results vary based on the study design and study group. Several factors affecting postoperative pain have been investigated, including working length determination [4], apical patency, various instrumentation or irrigation protocols

[5–8], number of visits [9], intracanal medicaments [10], root canal filling techniques [11, 12], and occlusion reduction [13]. Some of these studies compared post-obturation pain using the same root canal filling techniques but with different sealers in a single visit endodontic treatment [14–16]: cold lateral compaction using gutta percha cone with four different sealers (iodoform paste, Oxpara cement, eugenol-based sealer, and resin-based sealer) [14]; carrier base obturation using resinbased sealer and calcium silicate-based sealer [15]; and warm vertical compaction using gutta percha cone with three types of calcium silicate-based sealers [16]. There were no differences in postoperative sensitivity. In the investigations where filling techniques were the same between groups, the type of sealer did not influence postoperative endodontic pain [14–16].

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Tricalcium silicate-based hydraulic cements are gaining popularity in non-surgical root canal treatment [17]. Even though long-term clinical trials are lacking, silicate-based hydraulic cements have shown to be less cytotoxic compared to resin-based sealer in both ex vivo and animal models [18]. Premixed calcium silicate-based sealer has excellent physicochemical and biological properties, both with in vitro and in vivo animal studies, compared with conventional sealers [18]. Root canal filling with calcium silicate-based sealer and a single gutta percha cone using a sealer-based filling technique (SBT) is now widely accepted, with a 90.9% reported success rate in a retrospective clinical study at three years of observation [19].

A split-mouth clinical study showed that the incidence and intensity of postoperative pain were not statistically significant when comparing SBT and WVT with a resin-based sealer [20]. Similarly, a recently published randomized clinical trial using the two techniques to evaluate the postoperative pain after endodontic treatment had the same result [21]. However, the first investigation was conducted on previously endodontically treated teeth with asymptomatic apical periodontitis, while in the second trial, more than 85% of cases had a preoperative diagnosis of necrotic pulp. Thus far, there is no data on postoperative pain after treating teeth with irreversible pulpitis.

This prospective clinical trial aims to compare the postoperative pain after single-visit non-surgical root canal treatment of irreversible pulpitis cases using two root canal filling (RCF) techniques; SBT with gutta percha cone and calcium silicatebased sealer, and WVT with gutta percha cone and resinbased sealer. The null hypothesis was that there is no significant difference between the two groups.

Materials and methods

Study design and ethics

This prospective clinical trial was approved by the institutional review board (Protocol ID: 825494) and registered to ClinicalTrials.gov (NCT04462731). The subjects were recruited for the study from November 2016 to May 2018 at the Department of Endodontics, University of Pennsylvania, Philadelphia, Pennsylvania. All cases were treated by a first or second year postgraduate endodontic resident. Cases satisfying the inclusion/exclusion criteria were filled with either WVT using gutta percha with epoxy resin-based sealer (AH Plus Jet, Dentsply Maillefer, York, PA, USA) or SBT using gutta percha cone with premixed calcium silicate-based sealer (EndoSequence BC sealer, Brasseler, Savannah, GA, USA). The treatment groups were assigned to an experimental group by alternating months.

Sample size

The sample size was calculated based on a type I error of 0.05 and the power of 80%. The minimum sample size was determined to be 50 patients in each group. Twice the number of required cases were recruited in order to account for a high dropout rate for the patient population in West Philadelphia [20].

Subject enrollment and eligible criteria

This study was performed on teeth with irreversible pulpitis on patients at least 18 years old. Consecutive patients presenting to the Department of Endodontics for routine root canal treatment were recruited for the study. The patients had a noncontributory medical history (ASA class I/II). Included patients were given oral and written information about participation, and each signed the informed consent. The teeth were either asymptomatic or symptomatic irreversible pulpitis. The pulpal diagnosis was based on the clinical examination and confirmed upon accessing the teeth as per AAE consensus. Tenderness to percussion and palpation was also recorded as preoperative periapical symptoms. The following patients were excluded: patients under the age of 18, non-consenting to the study, medical history with ASA class III/IV, taking analgesics routinely for non-odontogenic reasons, and premedication with antibiotics or analgesics 24 h before the appointment. Teeth that were nonrestorable, periodontally involved (probing depth more than 4 mm), and presenting with periapical radiolucencies on the radiographs were also excluded.

Treatment protocol

After a thorough clinical and radiographic evaluation based on the inclusion/exclusion criteria, teeth satisfying the inclusion criteria were treated in a single visit. All teeth were isolated with rubber dam during root canal treatment. The procedures were performed under a microscope (OPMI Pico; Carl Zeiss, Gottingen, Germany). After access, location of canals, and determination of working length (WL) with Root ZX II apex locator (J Morita, Kyoto, Japan), the canals were instrumented using various 0.04 taper rotary NiTi instruments to a minimum apical size of 35. Four percent sodium hypochlorite was used as the main irrigant with a 31 gauge needle. Seventeen percent EDTA was used as the final irrigant. Passive ultrasonic irrigation (PUI) with a size 20 Acteon tip inserted 2 mm short of WL was performed with both 4% sodium hypochlorite and 17% EDTA for 10 s in each canal. After final irrigation, the canals were dried with paper points. The gutta percha master cone fit was verified with a periapical radiograph (Kodak RVG 6000, Carestream Dental, USA) before RCF.

Root canal filling techniques

The RCF techniques were dependent on the month the tooth was treated (alternating months). In the WVT group, teeth were filled with 0.04 taper gutta percha points (Meta Biomed Inc, Colmar, PA, USA) and AH plus sealer which was introduced with the master cone. A heated plugger (Alpha unit, B&L Biotech USA Inc, Bala Cynwyd, PA, USA) was placed within 3-5 mm of WL, and the remaining canal space was backfilled with additional sealer and thermoplasticized gutta percha using the beta unit (B&L Biotech USA Inc, Bala Cynwyd, PA, USA). In the SBT group, teeth were filled with BC sealer by injecting the sealer into the coronal third of each canal. Size 30 Lentulo spiral (Dentsply Maillefer, Tulsa, Oklahoma, USA) coated with additional sealer was introduced 3 mm short of WL depth at 300 rpm. Bioceramic coated gutta percha (EndoSequence BC points, Brasseler, Savannah, GA, USA) was dipped in BC sealer and introduced into the canal to WL. A heated plugger was used to sear the gutta percha point at each orifice. The coronal access cavities were filled with glass ionomer cement (Fuji IX, GC America, USA) as temporary restorative material.

Pain assessment

Patients were asked to rate the intensity of preoperative pain on a numeric rating scale (NRS) from 0 (no pain) to 10 (worst pain) before receiving root canal treatment. Along with NRS, the Wong-Baker facial grimace scale was also presented to the patients to help them in scoring the pain. At the end of the visit, the patients were given a survey and asked to rate the intensity of postoperative pain at 4, 24, and 48 h after the procedure. Patients were instructed to take 1000-mg acetaminophen as needed. If acetaminophen was consumed, the patients were asked to record the dose and time on the survey. They were provided stamped return envelopes to mail surveys back to the Department of Endodontics.

Statistical analysis

NRS scores between the RCF techniques were compared using Chi-square, *t*-test, or two-way ANOVA to explore the differences between the groups. Changes in outcome variables over time were compared by generalized estimating equation (GEE) analysis, which allows for correlation within repeated observations per individual [22]. Statistical analysis of the data was performed with IBM SPSS Statistics Version 23 (IBM Corp, Armonk, NY, USA). Statistical significance was set at 0.05 level.

Results

One hundred ninety-four surveys were given out in eighteen months. Ninety-two patients returned the survey, a 47% response rate, and all collected data were included for analysis. Figure 1 shows the flow chart of this study. All cases had adequate RCF level, with the gutta percha cone 0–2 mm within the canal from the radiographic apex. Sixteen endodontic residents provided the treatment, eight first year and eight from the second year. Thirty-eight percent of the irreversible pulpitis cases had no presentation of pain before treatment. The distribution of clinical features are listed in Table 1. Most of the distribution of the clinical features between the two RCF groups show no statistical differences ($p \ge 0.05$). However, the WVT group had significantly higher sealer extrusion cases than the SBT group (p = 0.004).

Periapical symptoms (percussion and palpation sensitivity), dental arch, provider experience, and RCF technique were not statistically related to postoperative pain ($p \ge 0.05$) (Table 2). Age, gender, presence of preoperative pain, root type, sealer extrusion, and assessment time point were significantly related to postoperative pain (p < 0.05). The NRS scores of gender and root type are presented in Figs. 2 and 3.

The RCF technique was not related to postoperative pain (p = 0.278). After root canal treatment, the pain levels reduced over time in both treatment groups. Although the WVT group had a significantly higher preoperative pain level than the SBT group (p = 0.01) (Table 3), the two RCF techniques had no statically significant differences in postoperative pain at all time points assessed (Fig. 4). The intensity of postoperative pain was similar in both groups when patients had preoperative pain. Those cases with no pain before the treatment had higher pain levels at 4 and 24 h postoperatively in the WVT group, but there are no statically differences with the SBT group.

The two RCF techniques showed similar pain levels postoperatively when there was no sealer extrusion (Fig. 5). In cases with sealer extrusion, the pain level gradually decreased but was still higher than those without extrusion. With sealer extrusion, postoperative pain was similar at 24 and 48 h postoperatively irrespective of the type of sealer.

Discussion

There are many variables in non-surgical root canal treatment that can contribute to postoperative pain [6-8, 10, 11].

In this study, all the providers followed the same biomechanical instrumentation protocol. They limited the treatment to a single visit to reduce the variation in treatment rendered, other than the obturation technique. According to the literature, postoperative pain declines significantly in the first 2 days after treatment [3]; hence, the observation time point in

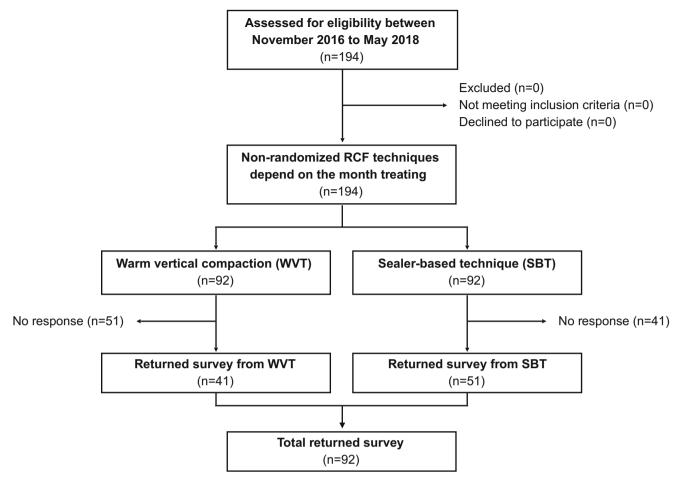


Fig. 1 Flow chart

Table 1 Clinical features distribution of patients included in this study

RCF technique	WVT	SBT	Total (%)	p Value
Mean age (year)	46.2 ± 14.4	46.7 ± 16.6	46.5 ± 15.6	0.88
Gender				
Female	30 (70.7%)	34 (66.7%)	64 (69.6%)	0.82
Male	11 (29.3%)	17 (33.3%)	28 (30.4%)	
Preoperative pain				
Yes $(VAS > 0)$	29 (29.3%)	28 (45.1%)	57 (61.9%)	0.14
No $(VAS = 0)$	12 (70.7%)	23 (54.9%)	35 (38.1%)	
Periapical symptoms				
Yes	17 (41.5%)	14 (27.5%)	31 (33.7%)	0.19
No	24 (58.5%)	37 (72.5%)	61 (66.4%)	
Location				
Maxilla	27 (65.9%)	33 (64.7%)	60 (65.2%)	1.00
Mandible	14 (34.1%)	18 (35.3%)	32 (34.8%)	
Root type				
Single-rooted	15 (36.6%)	18 (35.3%)	33 (35.9%)	1.00
Multi-rooted	26 (63.4%)	33 (64.7%)	59 (64.1%)	
Provider				
1st year residents	18 (43.9%)	27 (52.9%)	45 (48.9%)	0.41
2nd year residents	23 (56.1%)	24 (47.1%)	47(51.1%)	
Sealer extrusion				
Yes	17 (41.5%)	7 (13.7%)	24 (26.1%)	0.004**
No	24 (58.5%)	44 (86.3%)	68 (73.9%)	

Chi-square analysis and *t*-test were performed. Statistical significance: ** = p < 0.01

the current study was set at 48 h. Although the existence of preoperative pain is related to a higher incidence of postoperative pain [23], the current investigation that included both symptomatic and asymptomatic teeth found no significant impact of preoperative pain on postoperative pain after 48 h. Even though the pretreatment NRS scores and incidence of pain between the two groups are statistically different (p =0.01) (Table 3) (Fig. 4), the two obturation techniques have a similar postoperative pain level.

38.1% of cases with a preoperative diagnosis of irreversible pulpitis were asymptomatic before receiving endodontic treatment. The incidence of asymptomatic pulpitis is similar to that reported by Michaelson and Holland in a retrospective study (38.8%) [24]. Studies report that pain perception is gender-dependent due to biological mechanisms and sociocultural factors [25–28]. Men have a higher pain tolerance and are less likely to report pain compared to women. In this study, the response rate is much higher for the female group (Table 1). Also, the pain levels were higher in females at all assessed times (Fig. 2), which supports prior literature.

Traditionally, during obturation, the gutta percha acts as the primary core material, while the sealers seal the root canal space by filling gaps [29]. WVT is one of the most widely

 Table 2
 Generalized estimating

 equations analysis between
 postoperative pain and clinical

 features
 Features

Clinical features	Beta Coefficient	Standard error	95% confidence interval	p Value
Age (year)	-0.03	0.01	-0.05 to -0.01	0.005**
Gender	-0.58	0.30	-1.16 to -0.003	0.049*
Preoperative pain	0.22	0.06	0.11 to 0.33	< 0.0001****
Periapical symptoms	-0.12	0.38	-0.87 to 0.63	0.761
Location	-0.49	0.35	-1.18 to 0.21	0.169
Root type	0.77	0.39	0.01 to 1.53	0.048*
Provider	-0.31	0.34	-0.98 to 0.36	0.368
Obturation technique	-0.39	0.35	-1.07 to 0.31	0.278
Sealer extrusion	0.80	0.39	0.03 to 1.56	0.042*
Time	-0.03	0.004	-0.04 to -0.02	< 0.0001****

Gender: 0 = female, 1 = male. Preoperative pain: NRS from 0 to 10. Periapical symptoms: 0 = absent, 1 = present. Location: 1 = maxillary, 2 = mandible. Root type: 0 = single-rooted, 1 = multi-rooted. Provider: 1 = first-year resident, 2 = second-year resident. Obturation technique: 0 = WVT, 1 = SBT. Sealer extrusion: 0: absent, 1 = present. Statistical significance: * = p < 0.05, ** = p < 0.01, **** = p < 0.001

used obturation techniques. The primary aim of this technique is to increase the amount of gutta percha and reduce the amount of sealer within the root canal space. However, when heated gutta percha cools down, it shrinks significantly. Most sealers shrink upon setting and result in gaps between material and teeth [29–31]. AH plus sealer is a resin-based sealer reported to have the least amount of shrinkage [31]. Calcium silicate-based sealer has several properties that benefit endodontic obturation: hydrophilic, slight expansion while setting, and excellent biocompatibility [17, 31]. The contemporary concept of root canal filling has changed due to these properties of the calcium silicate-based sealer. Instead of gutta percha, the sealer is the main content of the root canal space and provides the seal. The gutta percha cone acts as the carrier to deliver calcium silicate-based sealer and provides the conduit to retreat the material if need be. Hence, the term sealerbased filling technique is more appropriate than "single cone technique."

Incidence of postoperative pain in relation to obturation technique on vital symptomatic teeth has been studied in one investigation [11], wherein obturation with Thermafil/backfill (Dentsply; York, PA) had significantly higher postoperative pain than those filled with Thermafil and cold lateral compaction. Atav et al. used the same RCF techniques: carrier-based

Fig. 2 The mean of NRS scores of gender at four time points (one preoperative and three postoperative). Subgrouped by the two RCF techniques. SBT, sealer-based filling technique; WVT, warm vertical compaction technique

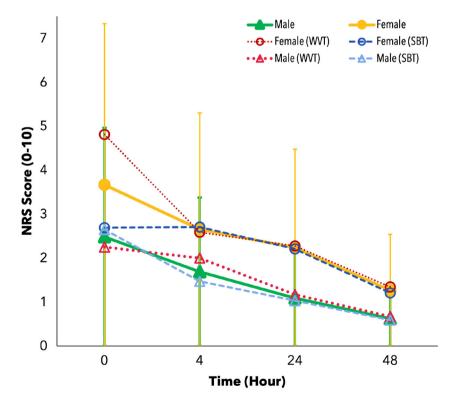
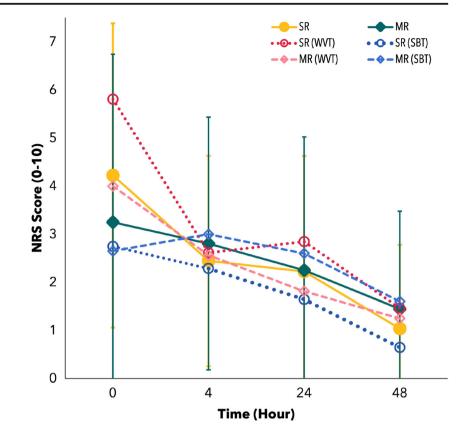


Fig. 3 The mean of NRS scores of root type at four time points (one preoperative and three postoperative). Subgrouped by the two RCF techniques. SBT, sealer-based filling technique; SR, single-rooted group; MR, multi-rooted group; WVT, warm vertical compaction technique



obturation with AH plus and BC sealer comparing the postoperative pain, which showed no difference between the two sealers in both vital and necrotic cases [15]. The obturation techniques in the two studies are not included in this investigation. One split-mouth clinical study compared postoperative pain using the same two obturation techniques and RCF materials in this study (WVT and SBT) and showed no difference [20]. A recent randomized clinical study also compared the postoperative pain with the two RCF techniques [21]. The split-mouth clinical study included previously endodontically treated teeth with asymptomatic lesions. The randomized clinical trial included normal pulp, pulpitis, and necrotic cases with 50% apical periodontitis cases. The current investigation evaluates pulpitis teeth with no apical lesions; however, all three studies agree that there are no statistical differences in postoperative pain with the two RCF techniques.

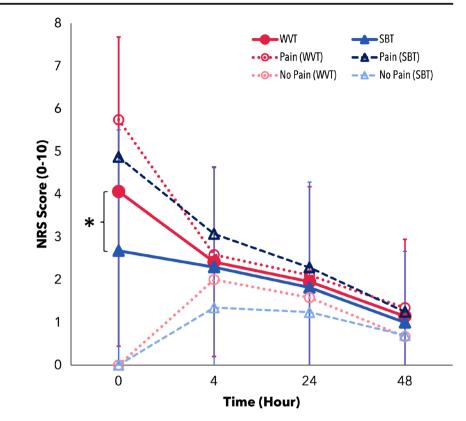
Sealer extrusion reportedly has no impact on endodontic outcomes [19, 32–34]; however, tissue reaction varies based on the type of sealer [35–37]. Recent case reports show that extruded calcium silicate-based sealer in contact with the

Table 3 Distribution anddescriptive statics of the meanvalue of NRS scores ofpreoperative pain andpostoperative pain after 4, 24, and48 h

Time	Mean NRS scores	95% confident intervention	Standard deviation	p Value
Preoperative	3.29	2.63-3.96	3.26	
WVT	4.06	2.95-5.17	3.62	0.01**
SBT	2.68	1.90-3.45	2.83	
Postoperative 4 h	2.35	1.88-2.81	2.28	
WVT	2.41	1.74-3.09	2.21	0.703
SBT	2.27	1.65-2.94	2.35	
Postoperative 24 h	1.88	1.39-2.36	2.35	
WVT	1.95	1.27-2.63	2.22	0.721
SBT	1.80	1.14-2.49	2.47	
Postoperative 48 h	1.07	0.71-1.42	1.71	
WVT	1.15	0.60-1.70	1.80	0.547
SBT	1.00	0.54–1.46	1.66	

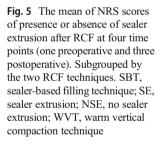
Analysis of variation (ANOVA) was performed. Statistical significance: ** = p < 0.05

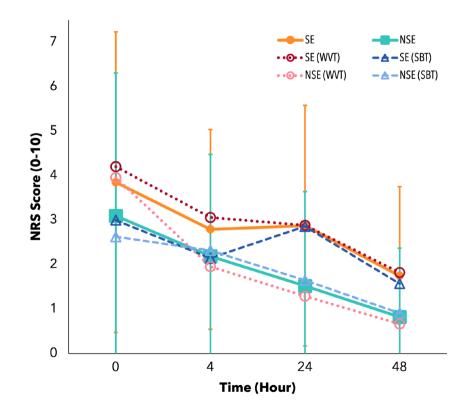
Fig. 4 The mean of NRS scores of two RCF techniques at four time points (one preoperative and three postoperative). Subgrouped by presence or absence of preoperative pain and the two RCF techniques. SBT, sealer-based filling technique; WVT, warm vertical compaction technique; *Statistically significant difference (p < 0.05)



periapical tissue has no foreign body nor inflammatory reactions in histological sections [37]. One in vivo histological study showed that the intraosseous tissue reacts similarly to

resin-based and calcium silicate-based sealers [38]. In the current investigation, there was a sealer extrusion in 41.5% of the WVT cases and 13.7% of the SBT cases. Another study





reported using SBT as RCF technique in previously endodontically treated teeth, and sealer extrusion was observed in 30.9% of the cases with normal apical tissue and 66.2% in cases with periapical radiolucency [19]. Another recent study reported sealer extrusion in 65% WVT and 49% SBT of cases diagnosed as necrotic pulp with apical periodontitis [21]. Less sealer extrusion in the current investigation could be attributed to all cases having no radiographic evidence of preoperative periapical radiolucencies. Histologic studies have showed that apical inflammatory root resorption can occur in 81% of cases when lesions are present and hence can contribute to inadvertent sealer extrusion [39]. Another factor could be that these were primary treatment cases, not retreatment, and hence had a lesser chance for over-instrumentation of the canals.

In this investigation, the pain levels after RCF decreased in all groups. Cases with sealer extrusion had higher levels of postoperative pain regardless of the RCF techniques used (Fig. 5). Although the two RCF techniques had no differences in postoperative pain, the WVT had a significantly higher chance to cause sealer extrusion than the SBT (Table 1). An in vitro study concluded that only low concentration AH plus sealer evoked calcitonin gene-related peptide (CGRP) release [40], while higher concentration AH plus sealer (set form) and the BC sealer (freshly mixed and set form) both inhibited the release of CGRP. The two sealers have the same performance after setting, which is 2.7 h for BC sealer and 11.5 h for AH plus sealer [31]. In the current data, the NRS scores were at the same levels in both SBT and WVT groups, at 24 and 48 h postoperatively, with sealer extrusion. Myles et al. reported that a change of 10-mm difference on the visual analogue scale is the minimum clinically significant difference, which is equivalent to a 1 point score difference on the NRS [41]. Sealer extrusion and gender had a similar difference of 1 on NRS scores. The postoperative pain increased as a result of sealer extrusion however was not dependent on the type of sealer used between the two groups.

Patient age was also shown to be related to postoperative pain in the current investigation, which corroborates with prior literature. The older patient population has a lower incidence of pain, both preoperatively and postoperatively [24]. The composition of human pulp tissue changes with age; pulpal tissue in young permanent teeth is highly innervated, and this innervation reduces with age [42, 43]. Degeneration of neural pulpal tissue is the probable cause for less sensitivity to pain in the older group.

The data set is representative of the patient population seen in an endodontic clinic. It includes both symptomatic and multi-rooted teeth with similar distribution in the two groups. This study revealed that preoperative pain level is significantly related to postoperative pain (p < 0.001), which agrees with previous investigations [23, 44]. The WVT group had fewer cases of asymptomatic pulpitis and a significantly higher pain level before treatment. Interestingly, the two RCF techniques still had similar postoperative pain levels at all assessed time points (Fig. 1). Preoperative tenderness to percussion and palpation was not a predictor for postoperative pain in irreversible pulpitis cases. Pain assessment studies generally limit their inclusion group to single-rooted teeth because multirooted teeth have a higher level of pain post treatment [20, 23]. However, since the purpose of this study was to compare the two RCF techniques in all root types, the root type was not limited to single or multi rooted teeth in this investigation.

All cases were treated by first and second year endodontic residents. First year residents are considered novice, still learning and improving their clinical skills to perform some of the RCF techniques. Residents' skills most likely improve over time as the result of the learning curve effect. The technical nature of clinical techniques requiring better skills and expertise can make randomization difficult [45]. Therefore, the treatment groups were assigned to an experimental group by alternating months for the entire month in a non-randomized interventional manner (quasi-experiment). Moreover, our results showed that the provider experience was not related to the postoperative pain of the two RCF techniques.

One of the limitations of the current study was the 47% recall rate. There are a few ways to conduct survey studies, including fax, mail, e-mail, and web-based approaches [46–48]; however, a study using a mixed-mode survey showed that mail surveys tend to be more effective than web-based or e-mail surveys [47]. A newer healthcare study also showed that the response rate administered by mail was superior to web-based surveys: 40% to 20%, respectively [48]. In this study, we provided stamped return envelopes to all patients, which might motivate them to return the survey. The study period was extended to 18 months instead of 1 year to allow a larger group of patients to be recruited. The loss to follow-up rate of the current data can potentially impact the results.

Conclusion

In this prospective clinical trial, root canal filling with sealerbased filling technique and warm vertical compaction technique did not affect the intensity of postoperative pain. Postoperative pain was related to age, gender, presence of preoperative pain, root type, and sealer extrusion, but not related to preoperative periapical symptoms, dental arch, and experience of the provider. Root canal filling with warm vertical compaction technique had a higher percentage of sealer extrusion than those with sealer-based filling technique in irreversible pulpitis cases.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the

institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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