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ORIGINAL RESEARCH

THE EFFECT OF DIFFERENT MODALITIES OF CRYOTHERAPY ON POST-OPERATIVE PAIN LEVEL IN PATIENTS WITH SYMPTOMATIC IRREVERSIBLE PULPITIS RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Background: The study aims to evaluate the effects of various cryotherapy treatment techniques on inflammatory mediators and post-operative pain.

Materials and Methods: The study was established on single rooted premolars exhibiting symptomatic pulpitis and apical periodontitis. 45 patients were assigned randomly into three groups (n = 15). The first group (the control group n=15) patients did not receive any cryotherapy treatment, The second group(n=15) submucosal infiltration of cold saline 2-5 °C was given before the treatment. The third group(n=15) intracanal irrigation of cold saline 2.5 °C was given after chemomechanical preparation. 3 samples were taken from the periapical fluid with paper point extended 2 mm past the apex as follows: the first sample was taken after pulp extirpation, the second sample was taken after mechanical preparation and the third sample was taken after 24 hours before obturation. The ELISA test was utilized to determine the substance P levels (neuropeptide released by nociceptors that plays a central role in initiating neurogenic inflammation). Taking analgesics with the existence, duration, and severity of pain using the VAS at 12,24 and 48 hours were recorded. Any adverse events were recorded. The data was examined using Friedman test and Kruskal Wallis test.

Results: The Second and The Third Group revealed a statistically significant variation having the lowest substance P level scores and the lowest post-operative pain in contrast with the control group that showed the highest level of substance p and post-operative pain ($P \leq 0.05$).

Conclusion: Submucosal and intracanal cryotherapy decreased substance p level and post-operative pain. This randomized, controlled, double -blinded, Phase IV clinical trial was registered at www.clinicaltrials.gov (NCT06090500). Date: 25/3/2024.

***Keywords*:** randomized clinical trials, symptomatic irreversible pulpitis, apical periodontitis, post-operative pain, substance P

INTRODUCTION

For a number of reasons, pain management during root canal treatment is an essential component of therapeutic practice. Firstly, patients generally anticipate a treatment experience that is free from pain or discomfort. Secondly, effective control of intraoperative pain can lessen postoperative symptoms[(1)] and make their management more straightforward. Lastly, a painful or unpleasant procedure may discourage patients from undergoing future endodontic treatments. Consequently, achieving a pain-free experience should be a fundamental goal for every dental practitioner.[(2)]

Postoperative pain (POP) continues to be a frequent concern among patients undergoing endodontic therapy with incidence rates between 3% and 58% reported.[(3)] Therefore, effective pain management is essential to obtaining a successful course of therapy outcomes. Several factors, including the intensity of preoperative pain, The state of the peri radicular tissues and the pulp, as well as radiolucency in the periapical region, can significantly influence the occurrence and severity of POP.[(4)] In addition, endodontic procedures may inadvertently induce chemical, mechanical, or microbial

irritation to the periapical tissues, which can further contribute to postoperative discomfort.[5] [6]

Cryotherapy, a therapeutic modality involving the application of cold, has become increasingly used because of its capacity to reduce pain, minimize swelling and lessen muscular spasms.[7] It provides analgesic benefits and minimizes tissue damage through a series of biophysical processes.[8] This effect is primarily achieved by decreasing metabolism of cells and oxygen intake, which therefore lowers the production of free radicals responsible for cell damage.[9]

It's interesting to note that when tissue temperature drops beneath a certain level (approximately 15 °C), a physiological response known as the "hunting reaction" occurs.[10] This process involves an initial phase of vasoconstriction followed by vasodilation, which is thought to contribute significantly to the therapeutic efficacy of cryotherapy in alleviating postoperative pain (POP).[10] Moreover, cooling slows down the conduction of nerve impulses [11]. At around 7 °C, the myelinated A-delta fibers—responsible for transmitting sharp, rapid pain sensations—cease to function completely [12], whereas the unmyelinated C-fibers, which convey dull and thermal pain sensations, become inactive at approximately 3 °C.[9]

Questionnaires and rating scales are frequently used in pain evaluation, with VAS being one of the most used techniques.[13] However, based on personal pain thresholds, these subjective measurements might differ.[14] Consequently, the use of objective biomarkers to supplement subjective evaluations has gained more attention. Interleukin-6 (IL-6), calcitonin gene-related peptide (CGRP), and substance P (SP) are examples of inflammatory cytokines and neuropeptides that are known to be important in the pathophysiology of endodontic discomfort and inflammatory conditions.[15] [9]

Substance P (SP) is a neuropeptide released by nociceptors that is essential for starting neurogenic inflammation.[16] Research has shown that higher substance P levels in the dental pulp are associated with increased neuronal activity.[16] Moreover, The amount of expression of substance P in pulpal nerves has been shown to dynamically alter in response to carious lesions, potentially influencing both Perception of pain and inflammatory processes.[16] Consequently, substance P may serve as a valuable biomarker for evaluating and contrasting pain intensity in patients following Endodontic therapy.[17] [6]The study's null hypothesis suggested that there wouldn't be any considerable variance among the values of inflammatory mediators between the control group and the group receiving cryotherapy. However, the study's findings revealed that cryotherapy led to a reduction in the levels of substance P, although This decline was not statistically significant. Despite the limited magnitude of change, the null hypothesis was ultimately rejected.[18]

The purpose of this study is to assess the effects of different modalities of cryotherapy treatment on post-operative pain and the level of inflammatory mediators.

MATERIALS AND METHODS

- **Research Ethics Approval and Clinical Trial Registration**

The study procedure and the informed consent form were accepted by the ethical committee of the Faculty of Dentistry at Ain Shams University. An FDASU-RecM012452 certificate of ethics committee approval issued January 31, 2024. Every patient filled out an informed consent form prior to participation. The time frame for conducting this study was April 1, 2024, to July 1, 2025. Registered under NCT06090500 on www.clinicaltrials.gov. at date 25/3/2024

- **Sample size calculation and power analysis:**

The sample size was determined based on an earlier study[19] as reference. This study found that a minimum sample size of 10 per group was acceptable, when mean ± standard deviation of group 1 was 4.08 ± 1.58 while estimated mean difference with group 2 was 2.5, when the power was 80 % & type I error probability was 0.05. The independent t test was performed by using P.S. power3.1.6. Total sample size increased to 15 per group to compensate for a 25 % drop out.

Total sample size = 15 per group

- **Patient selection:**

Eligibility criteria: patients referred to the Endodontics Department of October 6 University's Faculty of Dentistry in Giza, Egypt with pain brought on by irreversible pulpal inflammation with apical periodontitis from carious tooth that need root canal therapy were regarded as possible subjects for the study. Every participant in the study shared a few things in common. Each was an adult, over the age of eighteen, and each had a mandibular premolar with a single root—one small tooth that had become the source of intense and persistent pain. For many, the pain was relentless, sometimes intermittent but always sharp, refusing to fade even after the stimulus was gone Based on the clinical signs of significant preoperative pain (visual analogue scale) [VAS] > 7) and severe (VAS > 7).

Those with systemic diseases were excluded, as were teeth with open apices or any that had undergone root canal treatment before. The presence of sinus tracts or periodontal pockets deeper than 3 mm meant exclusion as well. Even recent use of antibiotics or painkillers within the past three days disqualified a patient.

Diagnostic confirmation:

The clinical diagnosis of symptomatic irreversible pulpitis with periodontitis at the apical region was determined based on an exaggerated response to the cold (Endo-Ice) pulp sensibility test and the existence

of copious pulpal bleeding upon access—confirming pulp vitality—along with a positive percussion response and normal periapical radiographic findings.

- **Study Design and Grouping:**

The Consolidated Standards of Reporting Trials statement CONSORT was followed in the design and reporting of this randomized, controlled, double-blind, Phase IV clinical trial.

Three groups of participants were established: Group I is the control group(n=15), Group II for submucosal cryotherapy(n=15) and Group III for intracanal cryotherapy(n=15). In The First Group (control group) patients were not treated with cryotherapy, In the Second Group submucosal infiltration of cold saline 2-5 °C was given before the treatment and in The Third Group intracanal irrigation of cold saline 2.5 °C was given after chemomechanical preparation.

- **Patients' randomization, allocation and blinding:**

Randomization and Allocation concealment: a total of 45 patients out of 90 who fulfilled the conditions were randomly distributed according to the ratio of 1:1:1 to each of the three treatment groups. Randomization was performed by using computer generated randomization software **(**www.random.org**)**.

An independent person randomly selected one envelope from a box when the patient arrived for treatment in order to place them in a particular group. The closed envelopes with information about the group members were kept closed until the cold application stage in order to prevent operator bias.

Sequence generation: for the patient's number was done using a random sequence number generated by computer software and a table kept with the co-investigator. For concealment, the group's name was written on a slip of paper and sealed inside a plain envelope.

Blinding: this study was double blinded for the patient and outcome assessor to minimize the assessment bias**.**

- **Treatment protocol and interventions:**

Group I (the control group): received the standard regimen for root canal therapy, which was carried out by a single endodontist for standardization. Pulp vitality was confirmed by bleeding during access cavity preparation under rubber dam isolation[20], following anesthesia with 1.8 mL of 4% articaine with a 1:100,000 dose of epinephrine by blockage of the inferior alveolar nerve. The working length was established to be 0.5 mm above the apex using an apex locator and radiographic verification. Root canal instrumentation was performed with PRO TAPER NEXT FILES using 2.5% NaOCl for irrigation and a final rinse of 17% EDTA, without activation techniques.[21]

Group II: Submucosal injection of 1 mL of 2 to 5°C cold saline infiltrated in the depth of the vestibule using a 1 ml of 31-gauge needle attached to an insulin syringe was given 5 minutes before anesthesia with a slow rate of injection 1 ml/30 seconds.[22] The cold saline syringe had previously been kept in the refrigerator, which had a digital water thermometer for temperature monitoring. The syringe was taken out of the refrigerator by the operator immediately before use.[23]

Group III: This group received 5 ml of 2.5 °C cold saline after mechanical preparation delivered 1mm shorter than the working length using NaviTip double Side-port(31gauge/27mm) gently without using force to apply the irrigant or agitation for 1 minute. The cold saline syringe had previously been kept in the refrigerator, which had a digital water thermometer for temperature monitoring. The syringe was taken out of the refrigerator by the operator immediately before use.[24]

Master cone will be checked radiographically, then, canal will be dried with absorbent paper points (Dent plus,korea). The root canal will then be filled using gutta percha matching master apical file with resin based sealer (Adseal-Meta Biomed-Korea) and auxiliaries.[25]

Evaluation of post-operative pain:

Patients were informed to record the existence and degree of postoperative pain at specific time intervals using the visual analogue scale (VAS) explained at the beginning of their visit. Pain assessments were recorded at 12, 24 and 48 hours after root canal obturation. Any postoperative flare-ups, such as intraoral or extraoral swelling, were also noted. All completed pain records were collected by a calibrated, physician who was blindfolded for analysis. Throughout the study, participants were advised to refrain from taking analgesics and to report any medication use, which would lead to exclusion from the study.[26]

Evaluation of substance P in apical fluid:

Periapical samples were collected using sterile paper points at three stages— Each sample was taken at different time intervals (T) The first sample was taken Upon completion of pulp extirpation(T1), The second sample was taken after completion of chemomechanical preparation and intracanal irrigation of the cold saline was given(T2) while the Third sample was taken after 24 hours before the obturation(T3). After drying the root canals, sterile paper points (size 20, taper of 6%) were inserted into the canal. These paper points were gently advanced approximately 2 mm into the periapical tissues past the apical foramen, where they were left in place for one minute to absorb the periapical interstitial fluid. Following collection, each paper point was sectioned 4 mm from its tip before further processing. Each cut paper point was placed into a 1 mL of phosphate-buffered saline (PBS, pH 7.4) in a 1.5 mL Eppendorf tube and subsequently kept at -80°C pending further investigation.[21]

Measurement of Substance P (SP) in apical fluid:
After collecting samples from all participants, the enzyme-linked immunosorbent assay (ELISA) technique was used to evaluate and identify the specimens. In the preanalytical preparation, to extract the supernatant from cellular debris, each sample was centrifuged for ten minutes at 4°C and 3000 rpm. Before being examined further, the resultant supernatant was carefully moved into sterile microcentrifuge tubes and kept at -80°C. Protein concentrations of each gingival crevicular fluid (GCF) sample were determined using the Bradford protein assay. Prior to ELISA, Phosphate-buffered saline (PBS) was used to dilute the samples at a 1:10 ratio.

Following the manufacturer's instructions, the prepared and diluted samples were placed to ELISA plates that had already been coated with capture antibodies and incubated. After incubation, unbound components were removed by washing, and detection antibodies were applied, and then the substrate solution was added after additional washing. A microplate reader was used to measure each well's optical density at 450 nm and these readings were used to determine SP concentrations in the samples.[21]

Harms

At the end of the trial, any adverse impacts seen in participants—whether in the intervention or control groups—were recorded and documented.

Management of these events was carried out as follows:

- Pain: Treated with anti-inflammatory analgesics (Brufen 600 mg, one tablet as required).
- Swelling: Addressed with warm compresses and warm saline mouth rinses. When fever or lymphadenopathy was present, antibiotics were prescribed (Augmentin 1 g, one capsule every 12 hours for 5 days).
- **Statistical methods and analysis:**

Statistical methods

Handling of numerical / quantitative variables:

Numerical data will be explored for normality by checking the data distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data will be presented as mean & standard deviation. If data will be normally distributed comparison between 2 different groups will be performed by using independent t-test, comparison between 2 related groups will be performed by using Paired t-test, while comparison between more than 2 groups will be performed by using One Way ANOVA test followed by Tukey`s Post Hoc test for multiple comparisons.

If data will be non-parametric data comparison between 2 different groups will be performed by using Mann-Whitney test, comparison between 2 related groups will be performed by using Wilcoxon Signed Rank test, while comparison between more than 2 groups will be performed by using Kruskal-Wallis test.

Handling of categorial / qualitative variables:

Data will be presented as frequency and percentages. All comparisons will be performed by using Chi square test.

Statistical analysis:

Graph Pad Prism, Windows Excel, and SPSS 27® (Statistical Package for Scientific Studies) were used for the statistical analysis. The Shapiro-Wilk and Kolmogorov-Smirnov tests for normality were used to examine the provided data, and the results showed that the data had a non-parametric origin.

As a result, the Kruskal Wallis test was used to compare various groups, followed by Duns Test for multiple comparisons, while the Friedman test and the Duns test for multiple comparisons were used to compare various time points. Using Spearman's Correlation coefficient, the relationship between substance P level and pain level was evaluated. A significance criterion of $p \leq 0.05$ was established.

RESULTS

Assessment of pain level:

Comparison between groups: (Figure 1)

After 12 hours, Group I (The control group) recorded the highest pain scores (4.93 ± 1.39), which were significantly greater than both Group II (submucosal infiltration of cold saline) (2.27 ± 1.44) and Group III (intracanal irrigation of cold saline) (2.00 ± 1.20) ($p < 0.0001$).

A similar pattern was observed **after 24 hours**, where pain levels decreased in all groups, but Group I (The control group) still demonstrated significantly higher values (3.07 ± 1.16) compared to Group II (1.33 ± 1.11) and Group III (1.13 ± 0.64) ($p < 0.0001$).

By 48 hours, pain intensity continued to decline, with Group I (the control group) maintaining higher scores with significant difference (1.47 ± 0.52) than Group II (0.80 ± 0.68) and Group III (0.53 ± 0.52) ($p < 0.0001$).

Table 1. comparison between groups regarding pain level at different time points:

N=15 / parameter	Group I		Group I I		Group I I I		P Value
	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	
After 12 hours	2.00/7.00 (5.00)	4.93 ± 1.39 a	0.00/5.00 (2.00)	2.27 ± 1.44 b	0.00/4.00 (2.00)	2.00 ± 1.20 b	<0.0001*
After 24 hours	1.00/5.00 (3.00)	3.07 ± 1.16 a	0.00/3.00 (2.00)	1.33 ± 1.11 b	0.00/2.00 (1.00)	1.13 ± 0.64 b	<0.0001*
After 48 hours	1.00/2.00 (1.00)	1.47 ± 0.52 a	0.00/2.00 (1.00)	0.80 ± 0.68 b	0.00/1.00 (1.00)	0.53 ± 0.52 b	<0.0001*

** Significant difference as P ≤ 0.05.*

Means with different superscript letters were significantly different as P ≤ 0.05.

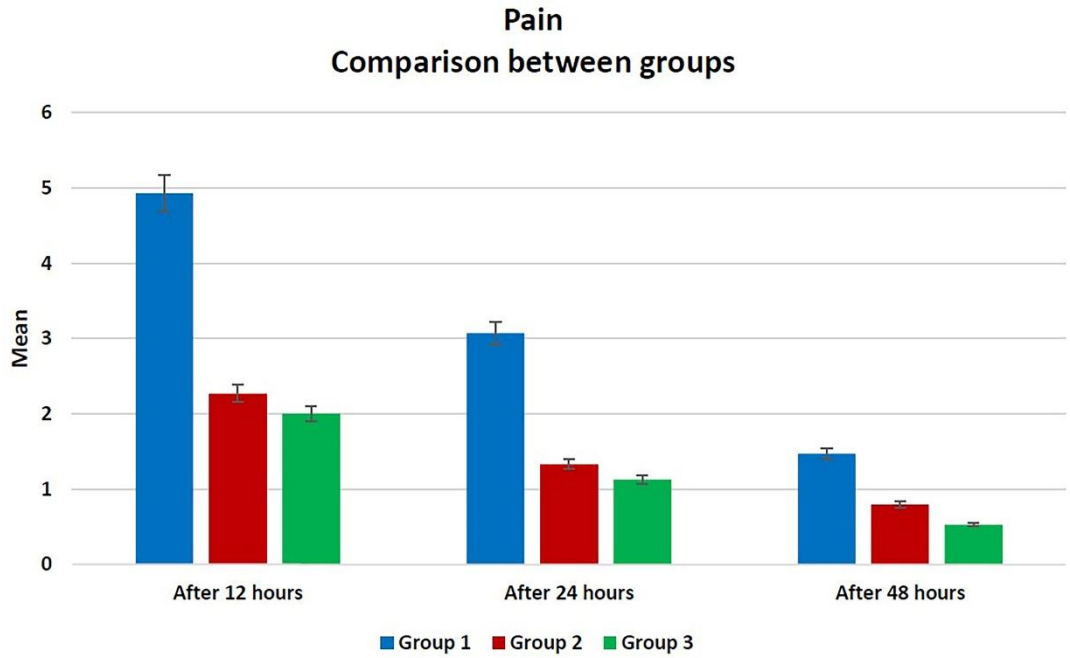

Figure 1. Bar chart showing Pain level at different time points.

Substance P level evaluation

Comparison between substance P level at different time points within each group: (Figure 2)

Comparison between groups was performed by using Friedman test which revealed a significant reduction in pain over time in all groups ($p < 0.0001$) as:

In Group I (the control group), Substance P levels showed a progressive and significant decline from T1(After pulp extirpation) (703.93 ± 86.84) to T2 (After mechanical preparation) (695.20 ± 85.84) and further to T3 (After 24 hours before obturation) (614.13 ± 115.22) ($p < 0.0001$).

Similarly, In Group II (submucosal infiltration of cold saline), Substance P levels decreased significantly over time, from T1 (400.73 ± 95.95) to T2 (290.33 ± 82.82) and T3 (254.40 ± 73.63) ($p < 0.0001$).

In Group III (intracanal irrigation of cold saline), a significant reduction was also observed between T1 (649.47 ± 75.39) and T2 (290.07 ± 31.39), with the levels remaining relatively stable between T2 and T3 (255.73 ± 126.60) ($p < 0.0001$).

Table 2. Comparison between substance P level at different time points within each group using Friedman test:

Parameter / N=15	T1		T2		T3		P Value
	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	
Group I	514 / 868 (705)	703.93 ± 86.84 a	501 / 855 (698)	695.20 ± 85.84 b	410 / 801 (619)	614.13 ± 115.22 c	<0.0001*
Group I I	298 / 615 (363)	400.73 ± 95.95 a	201 / 502 (262)	290.33 ± 82.82 b	191 / 487 (232)	254.40 ± 73.63 c	<0.0001*
Group I I	514 / 804 (660)	649.47 ± 75.39 a	219 / 349 (291)	290.07 ± 31.39 b	181 / 701 (220)	255.73 ± 126.60 b	<0.0001*

Means with different superscript letters were significantly different as $P \leq 0.05$.
*Significant difference as $P \leq 0.05$.

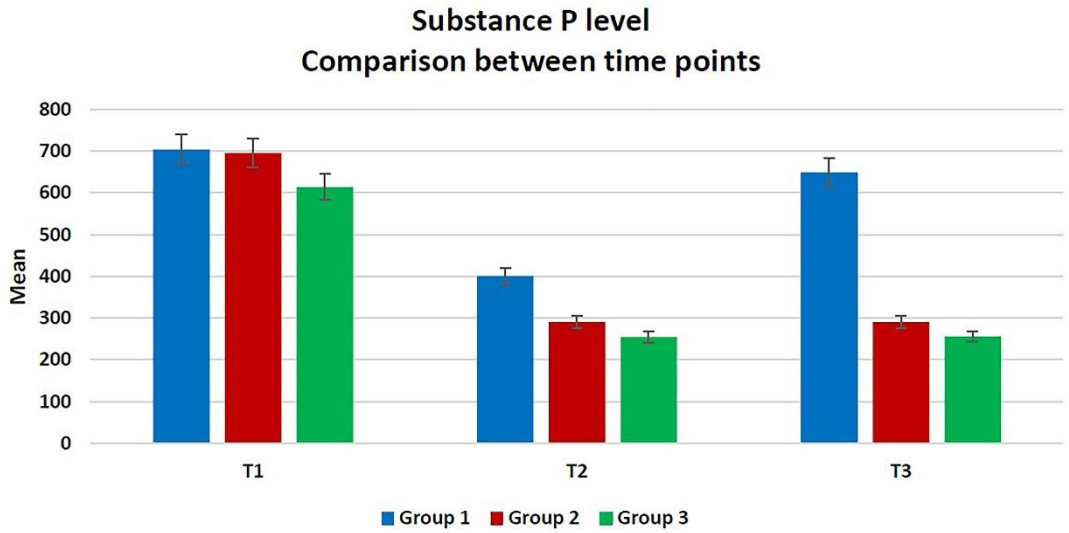

Figure 2. Bar chart showing substance P level at different time points within each group

Comparison between different groups: (Figure 3)
The Kruskal Wallis test was used to compare the groups, and the results showed that:
At T1 (After pulp extirpation), In Group I (The control group) recorded the highest Substance P level scores (703.93 ± 86.84), which were significantly greater than those of Group II (submucosal infiltration of cold saline) (400.73 ± 95.95) and Group III (intracanal irrigation of cold saline) (649.47 ± 75.39) ($p < 0.0001$).
At T2 (After mechanical preparation), Substance P levels decreased in all groups, but Group I still demonstrated significantly higher values (695.20 ± 85.84) compared with Group II (290.33 ± 82.82) and Group III (290.07 ± 31.39) ($p < 0.0001$).
At T3 (After 24 hours before obturation), levels continued to decline, with Group I maintaining higher scores (614.13 ± 115.22) than Group II (254.40 ± 73.63) and Group III (255.73 ± 126.60) ($p < 0.0001$).

Table 3. Comparison between groups regarding Substance P level at different time points:

N=15 / parameter	Group I		Group I I		Group I I I		P Value
	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	Min/Max (Median)	Mean ± SD	
T1	514 / 868 (705)	703.93 ± 86.84 a	298 / 615 (363)	400.73 ± 95.95 b	514 / 804 (660)	649.47 ± 75.39 a	<0.0001*
T2	501 / 855 (698)	695.20 ± 85.84 a	201 / 502 (262)	290.33 ± 82.82 b	219 / 349 (291)	290.07 ± 31.39 b	<0.0001*
T3	410 / 801 (619)	614.13 ± 115.22 a	191 / 487 (232)	254.40 ± 73.63 b	181 / 701 (220)	255.73 ±126.60 b	<0.0001*

**Significant difference as P ≤ 0.05.*
Means with different superscript letters were significantly different as P ≤ 0.05.

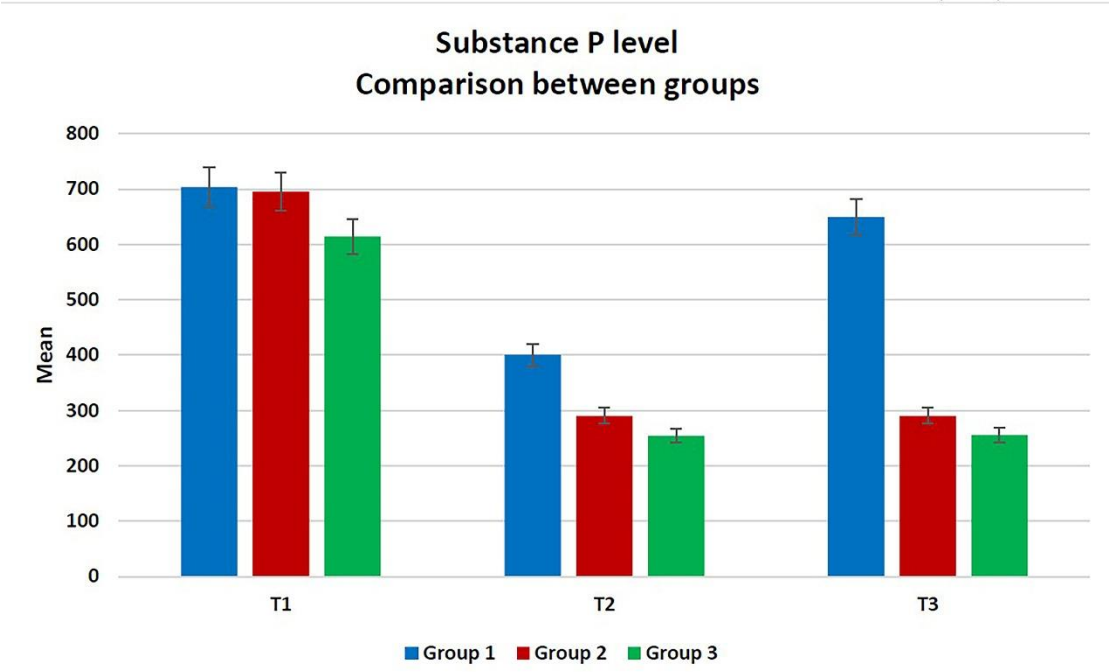

Figure 3. Bar chart showing substance P level at different time point

DISCUSSION

In endodontics, cryotherapy has been employed through several different methods. A commonly used technique is intracanal cryotherapy, which uses cold saline[27] as a final irrigant to lower the root's exterior surface temperature.[28] Additionally, cold compresses have been applied either intraorally or extra orally[29] following endodontic procedures to help reduce swelling and discomfort.[30] Cryotherapy has also been used post-surgically to manage inflammation and alleviate pain. However, a recent study introduced a novel technique involving injecting cold saline into the delicate tissues around the tooth that is causing symptoms. The research presented here is regarded as unique, as it explores a previously unexamined route of cryotherapy delivery within the context of endodontic pain management.[22]

The physiological impact of cold application can be explained by three primary responses: a reduction in cellular metabolic activity, vasoconstriction and suppression of neuronal receptors in the layer of skin and underlying tissues[18] . Furthermore, cryotherapy contributes to the reduction of edema formation by promoting sympathetically driven vasoconstriction, limiting the spread of inflammatory mediators to damaged tissues, and mitigating neurogenic inflammation through the suppression of sensory nerve activity. [21]

In our study, comparison between groups in assessment of pain level showed that:

After 12,24and 48 hours: The Second and The Third Group (cryotherapy different modalities) demonstrated reduced post-operative pain in comparison to the control group with significant difference. this was in accordance with Keskin and colleagues who demonstrated that patients who received cryotherapy with saline cooled to 2.5°C reported significantly less postoperative pain compared to those medicated at room temperature with saline.[26] Similarly, patients with pulp necrosis and symptomatic periodontitis at the apical region were examined by Vera et al. applying 2.5°C cold saline intracanal irrigation for five minutes, and found that this approach effectively reduced post endodontic pain.[27] Consistent with these findings, AlNahlawi and co-researchers observed that cold saline used because the final irrigant was more successful than saline at room temperature at reducing pain following treatment.[31] while the control group (Group I) revealed a considerably larger incidence of post-operative pain than both of the experimental groups at 12, 24, 48 hours following treatment. This outcome may be explained by potential irritation or trauma in the periapical tissues caused by the endodontic procedure, which could have triggered a localized inflammatory reaction resulting in post-endodontic discomfort that gradually subsided as the periapical region healed.[31]

Additionally, cold saline irrigation has been shown to lower the external root surface temperature by with this reduced temperature maintained for approximately four minutes more than 10°C.[19] Also this finding aligns with the conclusions of **Jain et al**.[24] who also advocated the exclusive use of cryotherapy to minimize post-operative discomfort in patients presenting with symptomatic irreversible pulpitis and periodontitis at the apical region.

The ongoing discussion about the impact of pulp state on post-operative pain outcomes is highlighted by Sipaviciute and Maneliene's report that between 47% and 60% of individuals with untreated necrotic pulp tissue suffered afterwards with pain despite the use of cryotherapy.[32]

Substance P (SP) plays a crucial role in modulating the inflammatory response by attracting and regulating various immune cells, including mast cells, lymphocytes, and macrophages. In addition to its cell-recruiting functions, SP also contributes to several other physiological processes, such as promoting chemotaxis, inducing vasodilation, enhancing immune system activity, and increasing plasma extravasation.[33] The dental pulp and periodontal tissue are known for having NKA, SP, and CGRP.[34]

In our study findings, At the time of taking the first sample (after pulp extirpation) T1: the control group(no cryotherapy treatment) and Group III (intra canal cold saline irrigation after mechanical preparation) showed higher levels of substance P Compared to Group II (submucosal cold saline infiltration) with significant difference and this is attributed to submucosal infiltration of cold saline was given before beginning of treatment before giving anesthesia so T1 in Group II showed this decline in substance P levels.[22]

At the time of taking the second sample (after mechanical preparation) T2 : Substance P levels in Group II and Group III were lower than those in the control group with significant difference as the intra canal irrigation of cold saline was given after mechanical preparation and immediately before taking the second sample that's why Group II and Group III showed this decline in substance P levels.[35]

At the time of taking the third sample (24 hours before obturation) T3: In comparison to the control group, Group II and Group III had reduced quantities of substance P with significant difference as the control group did not receive any cryotherapy treatment.[6]

Comparing substance P levels at different time points

with each group: it was found that In All Groups substance P levels at T1,T2 and T3 decrease gradually within time intervals with significant difference.[25] [22] [35]

Shalaby et al. [22] obtained similar results, showing that submucosal injection of cold saline (cryotherapy) successfully decreased the pulpal production of inflammatory mediators such Interleukin-6 (IL-6) and Substance P (SP).This approach was proposed as a safe, efficient, and cost-effective alternative to submucosal injections of anti-inflammatory medications for managing inflammation and controlling pain mediator release, thereby potentially reducing pain perception. Additionally, our results align with the research conducted by **Israa et al.** [21] which found that cryotherapy significantly decreased the concentration of Substance P in the apical fluid.

A study by **Reem et al.** [9] revealed that the most pronounced effects were found in the decreased post-operative pain scores and substance P concentrations, particularly when comparing the Cold irrigation group to the control group. These significant differences were both statistically and clinically relevant, reflecting a significant improvement in comfort for patients and reduction of neural inflammation. Overall, the results indicate that cold irrigation can effectively lessen the intensity and duration of pain after procedure, particularly in those with acute apical periodontitis.

The current investigation is subjected to several limitations. First, the sample size was relatively limited; a larger cohort would likely strengthen the statistical validity and enhance the external generalizability of the results. Second, the diagnostic criteria were confined exclusively to cases involving vital pulp. Future investigations should consider including both vital and necrotic (non-vital) pulpal conditions associated with apical periodontitis to present a more thorough overview of the spectrum of clinical care. Finally, the analysis focused solely on Substance P as an inflammatory mediator. The incorporation of additional neuro-inflammatory biomarkers in subsequent studies is recommended to allow a more thorough elucidation of the underlying pathophysiological mechanisms.

CONCLUSIONS

Cold saline intracanal irrigation and submucosal infiltration can both successfully lower post-operative pain and the level of inflammatory mediators such as substance P level. These results highlight the effectiveness of cryotherapy in modulating inflammatory mediators. Integrating these techniques into routine endodontic practice may enhance patient comfort. Cryotherapy therefore represents a practical and valuable adjunct for reducing postoperative discomfort.

DECLARATIONS

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Competing Interests

The authors have no competing interests to declare.

Informed Consent

Applicable

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