ASDJ
AINSHAMS DENTAL JOURNAL

Print ISSN 1110-7642
Online ISSN 2735-5039

AIN SHAMS DENTAL JOURNAL
Official Publication of Ain Shams Dental School
March2024 • Vol. 33

The effect of cryotherapy on substance P expression

Esraa Mohamed Ibrahim El Desouky Hamza[1], Tarek Mustafa Abd El Aziz[2], Maram Farouk Obeid[3]

Aim: In patients with symptomatic apical periodontitis, this randomized clinical trial evaluated the impact of intraoral cryotherapy treatment on the level of inflammatory mediators.

Materials and methods: Two groups, one for cryotherapy and the other for control (n = 10), were randomly assigned to twenty patients with symptomatic apical periodontitis. In the cryotherapy group, when biomechanical preparation was finished, 30 minutes of intraoral cryotherapy application (a cold pack of ice gel covered in a sealed plastic cover) were done. Samples of apical fluid were taken with paper points that extended 2 mm past the apex. In the control group after mechanical preparation and after 30 minutes, and in the cryotherapy group after mechanical preparation and after cold application. The ELISA test was used to measure the levels of substance P. The data were examined using an independent t-test.

Results: The cryotherapy group's change in substance P level was greater than that of the control group, but the difference was not statistically significant ($P \geq 0.05$).

Conclusions: Intraoral cryotherapy is an easy and affordable choice.

Keywords: Apical Periodontitis; cryotherapy; substance P

1. MSc Dept of Endodontics, Faculty of Dentistry, Ain Shams University, Cairo. Egypt.
2. Lecturer, Department of Endodontics, Faculty of Dentistry, Ain Shams University.
3. Professor, Department of Endodontics, Faculty of Dentistry, Ain Shams University.

Corresponding author: Esraa Mohamed Ibrahim El Desouky Hamza, email: hamzaesraa1993@gmail.com

Introduction

A substantial part of the inflammatory process is played by substance P. It is released by neurons in reaction to a range of harmful events.[1] Cryotherapy is one of the indicated treatments. The therapeutic procedure of reducing tissue temperature is known as "cryotherapy". The Greek words "cryos" and "therapeia," which translate to "cure" and "cold," respectively, are the source of the name.[2] It has mostly been used to treat lower back pain, runner's knee, tendonitis, sprains, arthritis pain, pains, and swelling, as well as pain or swelling under a cast or splint following hip or knee replacements.[3] Cryotherapy has, nevertheless, been used in dentistry to treat temporomandibular joint issues associated with edema, discomfort, and arthritis following intraoral surgical procedures such as implant insertion, extractions, and periodontal surgery.[4]

A new study conducted[5] revealed that the external root surface temperature was significantly lowered by over 10°C and maintained long enough to possibly have a local anti-inflammatory effect in the periradicular tissues through intracanal delivery of a cold saline solution (2.5°C) with negative pressure irrigation. In reference to the previous findings, no research has examined the therapeutic effects of this newly implemented approach on substance P levels. In this study, the effect of intraoral cryotherapy on inflammatory mediator (substance P) levels in patients with symptomatic apical periodontitis is compared.

Materials & Methods

I-Patients selection:

Twenty patients were chosen from the Department of Endodontics' outpatient clinic at Ain Shams University's Faculty of Dentistry in Cairo, Egypt.

Inclusion criteria: more than eighteen years old. possessing a mandibular premolar tooth with a single root and a vital pulp. Based on the clinical manifestations of significant preoperative pain (visual analogue scale [VAS] > 7) and severe percussion pain (VAS > 7), these individuals were diagnosed with apical periodontitis.

Exclusion criteria: the existence of allergic responses or other systemic diseases. use of antibiotics or analgesics within three days. severe periodontal condition. In the investigated tooth, there are periodontal pockets larger than 3 mm. Lack of bleeding during access cavity preparation in the pulp chamber Issues in measuring the working length. Broken files, over instrumentation, and overfilling or incomplete filling.

The study procedure was approved by the ethical committee of the Faculty of Dentistry at Ain Shams University. An FDASU-RecIM012117 certificate of ethics committee approval issued January 20, 2021. Every patient signed an informed consent form prior to participation. The time frame for conducting this study was September 1, 2022, to February 1, 2023. Registered under NCT06082479 on www.clinicaltrials.gov.

II.Study Design and Grouping:

This research was designed to be a single-center, Phase IV clinical trial that was randomized, controlled, and single-center. Two groups of participants were established: After the completion of the mechanical preparation, Group I (cryotherapy) (n = 10) cryotherapy was applied. Group II (control) (n = 10) got a normal root canal treatment without using cryotherapy of any kind.

A) A random sequence number produced by computer software was used to generate the sequence.

B) Allocation Concealment Mechanism: Patient coding was contained in carefully sealed envelopes holding folded and numbered papers.

III. Treatment protocol and interventions:

Patients who fulfilled the requirements for inclusion had their age and gender recorded. A lone endodontist treated every patient in the trial. When the access cavity was being prepared, pulp vitality was verified by pulp bleeding. All of the teeth were anaesthetized with an injection of an inferior alveolar nerve block. One carpule of anesthetic solution containing ARTINIBSA 4%:100,000 epinephrine (Inibsa, Spain) 1.8 mL was given to each patient. Supplemental anesthesia was administered when it was required. Rubber dam isolation was used during the access cavity preparation process. To achieve the initial glide path, a size 10 K-file (Mani, ICN, Japan) was introduced into the canal. The electronic apex locator, EPEX (China), was used to calculate working lengths.

A working length that was 0.5 mm shorter than the total length was chosen. Periapical radiography validated the working length's accuracy. Using M PRO FILES (China), root canals were created in accordance with the manufacturer's guidelines. Two milliliters of 2.5% NaOCl were used to irrigate the root canals following the preparation of the coronal thirds of the canals. 2.5% NaOCl was used in two milliliters for each pecking (in-out) motion. First, 5 mL of 2.5% NaOCl and then 5 mL of 17% EDTA (META BIOMED, Korea) were added and left for a minute each. There was no application of irrigation activation methods.

Sample collection: Following the completion of the chemomechanical preparation, the first sample was taken. After the root canals were dried, three series of paper points #25 were inserted into them. These were then soaked in the periapical interstitial fluid for a minute after being inserted two millimeters past the apical foramen into the periapical tissues. Cut each paper point four millimeters from the tip, drop each into a 1.5 mL Eppendorf tube (Swanscombe, UK) filled with 1 mL of phosphate-buffered saline (pH 7.4), and store at -80°C. As with the first sample, an apical fluid sample was obtained from the test group for the second sample following intraoral cold treatment. A second sample was collected from the control group following a 30-minute chemomechanical preparation period.

Cold application: On the vestibular surface of the treated tooth, an intraoral cold pack, measuring 2.5 x 5 cm and covered in a sealed plastic cover, was inserted. For ten minutes, each cold pack was maintained. Each patient received three packets for a total of thirty minutes. In the event that the patient experienced an intense cold or burning, they were told to take the cold pack off for one to two minutes. A digital thermometer (Brannan, UK) was used to measure the gel's temperature after it was taken out of the freezer and placed in an ice box (Cosmoplast, UAE) with a cooling gel inside (Exam Packaging, Belgium). The master cone underwent radiographic inspection. Next, gutta-percha (Meta Biomed, Korea) and a resin-based sealer (Adseal, Meta Biomed, Korea) were used to fill the root canal. Using glass ionomer filling (Fuji, Japan), the cavity was sealed.

Enzyme-linked immunosorbent assay test: Following the collection of samples from each patient, the ELISA method was used to assess the samples.

1. Methods

1.1. Preanalytical periapical fluid preparation

A. Centrifugation: The specimen was collected and centrifuged at 4°C for 10 minutes at 3000 rpm.

B. Aliquoting: The supernatant was carefully transferred to a fresh, sterile microcentrifuge tube after centrifugation, and it was stored at -80°C until it was needed.

C. Protein estimation: A Bradford protein assay (catalogue number B6916; Sigma-Aldrich, Saint Louis, USA) was used to assess the protein content of the GCF sample.

D. Dilution: PBS (phosphate-buffered saline) was used to dilute the sample 1:10.

1.2. Measurement of SP in apical fluid

The ELISA test can now be performed on the prepared apical fluid sample. The SP (Substance P) ELISA kit, cat no. E-EL-0067, from Elabscience Biotechnology, China, was used to conduct the test. After adding the diluted sample, the ELISA plate covered with the capture antibody was allowed to incubate for a predetermined amount of time. After washing the unbound material off the plate, the detection antibody was applied. Following washing and incubation, the substrate was added, and a spectrophotometer (ELx 800; Bio-Tek Instruments Inc., Winooski, VT, USA) with a microplate reader was used to measure the absorbance at 450 nm.

Statistical analysis

Categorical data, which was in the form of frequency and percentage values, was analyzed using Fisher's exact test. They provided numerical data, as shown for the mean and standard deviation (SD). Shapiro-Wilk's test and an examination of the data distribution were used to determine whether they were normal. Using an independent t-test, normally distributed data (age and substance P) were examined. A significance level of $p<0.05$ was applied.

Results

Twenty cases, ten cases each, were randomly and equally distributed across the tested groups. In the cryotherapy group, there were two males and eight females, compared to three males and seven females in the control group. The control group's mean age of the cases was (29.70±6.29) years, while the cryotherapy group's mean age was (25.15±5.43) years. Pre-operative pain on percussion and VAS mean values were(9.20±0.92) and (9.30±0.82) in the control group and (9.20±0.79) and(9.50±0.85) in the cryotherapy group, respectively. When it came to various demographic data and baseline characteristics, there was no discernible difference between the two groups ($P > 0.05$).

The substance P level (pg/ml) of the cryotherapy group was found to be higher than that of the control group; however, the difference was not statistically significant ($p > 0.05$). Figure (1) and Table (1)

Table (1): Intergroup comparisons, mean and standard deviation values of difference in substance P level (pg/ml) for different groups

Difference in substance P level (pg/ml) (mean±SD)		p-value
Control	Cryotherapy	
55.77±18.59	60.06±8.76	**0.518ns**

***; significant ($p \leq 0.05$) ns; non-significant (p>0.05)**

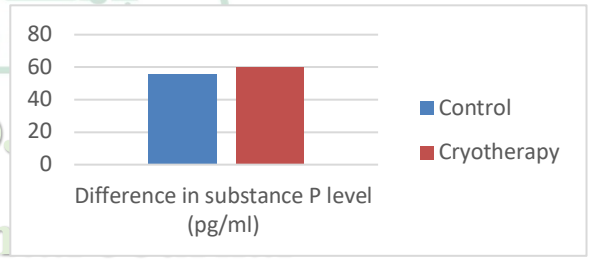

Figure (1): Bar chart showing mean and standard deviation values of difference in substance P level (pg/ml) for different groups.

Discussion

According to the null hypothesis, the control group and the cryotherapy groups would not have different levels of inflammatory mediators. The outcomes of the investigation showed that substance P was reduced by cryotherapy. Still, the change was not great enough to be significant. Consequently, the null hypothesis of the

investigation was rejected. Three basic physiological reactions might occur from applying cold: a decrease in metabolic activity, suppression of neural receptors in the skin and subcutaneous tissues, and vasoconstriction.[2] Furthermore, cold therapy decreases the generation of edema through sympathetically mediated vasoconstriction, the dissemination of inflammatory mediators to wounded tissues, and neurogenic inflammation as a result of decreased neuronal activity in sensory nerves.

Secondary hypoxia damage is lessened when wounded tissues have a lower metabolism.[6] Cold therapy lowers skin temperature to a depth of 2 to 4 cm, which decreases tissue nociceptors' activity and causes cold-induced neurapraxia, a slowing of conduction along peripheral axons [7]. Cryotherapy inhibits nociception in the spiral cord by activating thermoreceptors, which are temperature-sensitive nerve endings that are triggered by changes in tissue temperature. [6] The study's conclusion may be explained by the fact that, as Jorge Vera et al.'s 2015 in vitro investigation demonstrated, the intraoral cold application lowered the root's surface temperature. [5]

It was proposed that a localized anti-inflammatory impact in the periapical tissues could be induced by this drop in temperature. Nevertheless, the first clinical research utilizing this idea was conducted by Keskin et al. [8], In comparison to the control group, the study's findings demonstrated that applying a final irrigation of 2.5°C cold saline for five minutes decreased postoperative pain. They did not, however, differentiate between pulpitis that was asymptomatic and that was symptomatic, nor did they discriminate between cases of apical periodontitis and those without it. Intracanal cryotherapy was found by Bazaid et al. [9] to alleviate pain in patients with apical periodontitis and symptomatic irreversible pulpitis. Cryotherapy did not work in previously asymptomatic cases without periapical pathosis, as noted by Alharthi et al.[10]

This was in line with the recommendation made by Jain et al.[11], who also recommended utilizing cryotherapy entirely to lessen postoperative discomfort in cases of symptomatic irreversible pulpitis with apical periodontitis. Regarding the 30-minute application time, we adhered to the same methodology as Gündodu & Arslan's (2018) clinical trial.[12] Instead of using messy, non-standardized ice in gauze, we utilized ice gel covered in a plastic cover, and we replaced it every ten minutes because, in clinical procedures, cold solutions applied to teeth should quickly warm up to body temperature. Our study's findings are consistent with this one.In our investigation, the level of substance P in the apical fluid was reduced by cryotherapy. Still, the change was not great enough to be noteworthy. The small sample size of the study and variations in the ELISA kits' sensitivity could be the cause of these results. Additionally, if the periapical fluid samples used to test neuropeptide levels were below the necessary level, it's probable that the results might be negatively impacted. In this study, three series of paper points #25 were inserted in accordance with Hazal BC AKC[13], passing 2 mm beyond the apical foramen into the periapical tissues. As a benchmark, these paper points were soaked in the periapical interstitial fluid for one minute. Similar to the research conducted by Emad A et al. (2021)[14], Emad A et al. (2021)[15] and Keskin et al. (2023)[16].

Periodontal ligament inflammation has a neurogenic basis similar to that of the dental pulp and is brought on by the release of neuropeptides from injured periapical tissue C-type nerve fibers during root canal therapy.[17]

Substance P (SP) can recruit and control inflammatory cells, such as mast cells, lymphocytes, and macrophages. It can also

have other effects such as chemotaxis, vasodilation, immune system activation, and plasma extravasation.[1] NKA, SP, and CGRP are known to be present in the pulp and periodontium.[18] Certain A delta fibers and unmyelinated C fibers contain these neuropeptides. SP and NKA are released in pulp injuries when C-type nerve fibers are activated and bradykinin and prostaglandins are present in the tissue.[19] SP may enhance the synthesis of cytokines and arachidonic acid[20] metabolites, as well as macrophage chemotaxis and phagocytosis.[21]

The literature contains no prior research on the impact of intraoral cryotherapy on substance P levels. As such, a direct comparison is not possible. Although they employed a different mediator, recent clinical research by Emad et al. (2021) [14], with results comparable to ours, found that intracanal cryotherapy decreased the amount of IL-6 in the periapical exudate in comparison to the control group. There was no discernible statistically significant difference between the groups. Keskin et al. (2023) [16] discovered that the levels of IL-8, TNF-α, PGE2, and MMP-8 in the cryotherapy and control groups reduced with time, although the decline was not statistically significant. These results are similar to our study's findings.

Conclusion

Within the confines of the current investigation, the following result was discovered: Although there was no statistically significant difference between the two, substance P levels were lower in cryotherapy samples than in control samples.

References

1- Caviedes-Bucheli J, Muñoz HR, Azuero-Holguín MM, Ulate E. Neuropeptides in dental pulp: the silent protagonists. J Endod. 2008 Jul;34(7):773-88.
2- Modabber A, Rana M, Ghassemi A, et al. Three-dimensional evaluation of postoperative swelling in treatment of zygomatic bone fractures using two different cooling therapy methods: a randomized, observer–blind, prospective study. Trials 2013;14:238.
3- Higgins TR, Heazlewood IT, Climstein M. A random control trial of contrast baths and ice baths for recovery during competition in U/20 rugby union. J Strength Cond Res 2011;25:1046–51.
4- Laureano Filho JR, de Oliveira e Silva ED, Batista CI,Gouveia FM. The influence of cryotherapy on reduction of swelling, pain and trismus after third-molar extraction:a preliminary study. J Am Dent Assoc 2005; 136: 774-8.
5- Vera J, Ochoa-Rivera J, Vazquez-Carcaño M, Romero M, Arias A, Sleiman P. Effect of Intracanal Cryotherapy on Reducing Root Surface Temperature. J Endod. 2015 Nov;41(11):1884-7.
6- Nadler SF, Weingand K, Kruse RJ. The physiologic basis and clinical applications of cryotherapy and thermotherapy for the pain practitioner. Pain Physician 2004;7:395–9.
7- Malanga GA, Yan N, Stark J. Mechanisms and efficacy of heat and cold therapies for musculoskeletal injury. Postgrad Med. 2015 Jan;127(1):57-65.
8-Keskin C, Ozdemir O2, Uzun I, GUler B. Effect of intracanal cryotherapy on pain after single-visit root canal treatment. Aust Endod J 2017;43:83–8.
9-Bazaid DS, Kenawi LMM. The effect of intracanal cryotherapy in reducing postoperative pain in patients with irreversible pulpitis: a randomized control trial. Int J Health Sci Res. 2018; 8(2):83-88.
10- Alharthi AA, Aljoudi MH, Almaliki MN, Almalki MA, Sunbul MA. Effect of intra-canal cryotherapy on post-endodontic pain in single-visit RCT: A randomized controlled trial. Saudi Dent J. 2019 Jul;31(3):330-335.
11- Jain A, Davis D, Bahuguna R, Agrawal A, Singh S, Ramachandran R, Varghese A. Role of cryotherapy in reducing postoperative pain in patients with irreversible pulpitis; an in-vivo study. Int J Den Med Sci. 2018;2(1):43-9.
12- Gundogdu EC, Arslan H. Effects of Various Cryotherapy Applications on Postoperative Pain in Molar Teeth with Symptomatic Apical Periodontitis: A Preliminary Randomized Prospective Clinical Trial. J Endod. 2018 Mar;44(3):349-354.
13-Bıçakcı H, Çapar İD, Genç S, İhtiyar A, Sütçü R. Influence of Rotary Instrumentation with Continuous Irrigation on Pain and Neuropeptide Release Levels: A Randomized Clinical Trial. J Endod. 2016 Nov;42(11):1613-1619.
14- Emad A, Abdelsalam N, Fayyad D. Influence of intracanal cryotherapy on postendodontic pain and interleukin-6 expression using different irrigation protocols: A randomized clinical trial. Saudi Endodontic Journal. 2021 May 1;11(2):246-.

15-Emad A, Abdelsalam N, Fayyad D. Influence of intracanal cryotherapy on inflammatory mediators expression using different irrigation protocols: a randomized clinical trial. Dental Science Updates. 2021 Sep 1;2(2):165-74.
16- Keskin C, Aksoy A, Kalyoncuoğlu E, Keleş A, İlik AA, Kömeç O, Yüzgüleç E, Akgün H, Alak SG, Tokur O. Effect of intracanal cryotherapy on the inflammatory cytokine, proteolytic enzyme levels and postoperative pain in teeth with asymptomatic apical periodontitis: A randomised clinical trial. International Endodontic Journal. 2023 May 24.
17- Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 1998;9:498–52.
18- Ohkubo T, Shibata M, Yamada Y, et al. Role of substance P in neurogenic inflammation in the rat incisor pulp and the lower lip. Arch Oral Biol 1993;38:151.
19-Caviedes-Bucheli J, Correta-Ortiz JA, Garcia LV, et al. The effect of cavity preparation on substance P expression in human dental pulp. J Endod 2005;31:857–9.
20- Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988;241:1218–21.
21- Ruff MR, Wahl SM, Pert CB. Substance P receptor-mediated chemotaxis of human monocytes.Peptides 1985;6:107–11.