

## THE EFFECT OF DIFFERENT INSTRUMENTATION KINEMATICS ON THE CHANGE IN SUBSTANCE P LEVELS IN PATIENTS WITH SYMPTOMATIC APICAL PERIODONTITIS. A RANDOMIZED CONTROLLED TRIAL

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### ABSTRACT

**Aim:** This study aimed to assess the influence of continuous rotation and reciprocation kinematics on the change in substance P (SP) levels in patients diagnosed with irreversible pulpitis and symptomatic apical periodontitis (SAP).

**Materials and methods:** A total of twenty patients were randomly assigned into two groups: Continuous Rotation Group (CG) (n=10), where mechanical preparation was performed with the EdgeEndox7 rotary system (Albuquerque, NM, USA), and Reciprocation Group (RG) (n=10), with EdgeOne Fire reciprocating system (Albuquerque, NM, USA). Apical fluid (AF) samples were collected and changes in SP levels were quantified through ELISA. Data were statistically analyzed utilizing the independent t-test, Friedman's test, and Nemenyi post hoc test.

**Results:** The difference of SP levels in the reciprocating group was significantly higher when compared to the continuous rotation group ( $P \leq 0.05$ ) when measured preoperatively and at the beginning of the second visit.

**Conclusion:** Our findings suggest that continuous rotation kinematics lead to a reduction in substance P levels.

**KEYWORD :** Continuous rotation, reciprocation, substance P, apical periodontitis

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## INTRODUCTION

The complete removal of microbes, microbial toxins, and pulp tissue from the root canal system is essential to the effectiveness of root canal therapy<sup>1</sup>. This is accomplished through the chemo-mechanical preparation of the root canal. Hand devices made of stainless steel were used in the past to shape root canals, but their limited flexibility resulted in iatrogenic mistakes like canal transportation and ledges<sup>2</sup>. By dramatically lowering such errors and improving preparation safety, the advent of nickel-titanium engine-driven files has fundamentally changed this procedure<sup>3</sup>.

With a primary focus on optimizing flexibility and reducing fractures through unique modifications, manufacturers have improved file design, metallurgy, and machining techniques over time<sup>4</sup>. Henry Schein Dental has released the EdgeEndoX7 system (EdgeEndo, Albuquerque, NM, USA), which combines “Canal Contouring Technology” with the exclusive FireWire™ heat-treatment technology. This technology is said to improve file flexibility and lower the restoration force associated with other NiTi files<sup>5</sup>. This system’s files have a triangular cross-section, a continuous 0.04 taper, and a changeable helix angle<sup>6</sup>.

A recent proposal involves changing the kinematics of the instruments from continuous rotation to alternate clockwise and counterclockwise reciprocating motion to reduce torsional loads and improve fracture resistance<sup>7</sup>. Using FireWire™ technology, Henry Schein Dental launched the EdgeOne Fire reciprocating system (EdgeEndo, Albuquerque, New Mexico, USA), which features a parallelogram cross-section, two cutting edges, and an off-center design<sup>8</sup>. The cutting and releasing angles are used in a reciprocating fashion, involving a change in rotational direction<sup>9</sup>

It is unavoidable for bacteria, pulp tissue, dentinal chips, and irrigants to be extruded peri-apically during root canal preparation<sup>10</sup>, and strong evidence

supports the direct link between instrument motion and debris extrusion<sup>11</sup>. Unfortunately, postoperative pain (POP) is a substantial difficulty resulting from these extrusions, creating a significant dilemma for both patients and dentists<sup>12</sup>.

Several studies have found a link between POP levels and neuropeptide synthesis by sensory pulpal neurons in response to damaging stimuli. For example, afferent fibers (nociceptors) produce substance P (SP), which causes neurogenic inflammation and irreversible pulpitis<sup>13</sup> and can be used to validate and compare POP<sup>14</sup>.

Randomized clinical trials are required to analyze clinical outcomes to strengthen evidence-based results. The goal of this study was to see how SP levels changed after using the EdgeEndoX7 and EdgeOne Fire systems with various kinematics in patients with irreversible pulpitis in mandibular second premolars with symptomatic apical periodontitis (SAP). The null hypothesis states that the choice of preparatory kinematics does not affect SP level variations in SAP patients.

## MATERIALS AND METHODS

### I- Study Design and setting

This is a single-blinded, double-arm, randomized controlled clinical trial that was designed and reported in accordance with the Consolidated Standards of Reporting Trials statement CONSORT<sup>15</sup>. A flow diagram representing the Consolidated Standards of Reporting Trials of the study is shown in (Fig. a). The study participants were drawn from the outpatient Endodontic clinic at Ain Shams University’s Faculty of Dentistry. Following a thorough explanation of the study’s purpose, methods, benefits, and potential risks, all applicants signed a written consent form.

### II- Sample size calculation and power analysis:

A power analysis was designed to have adequate power to apply a two-sided statistical test of the

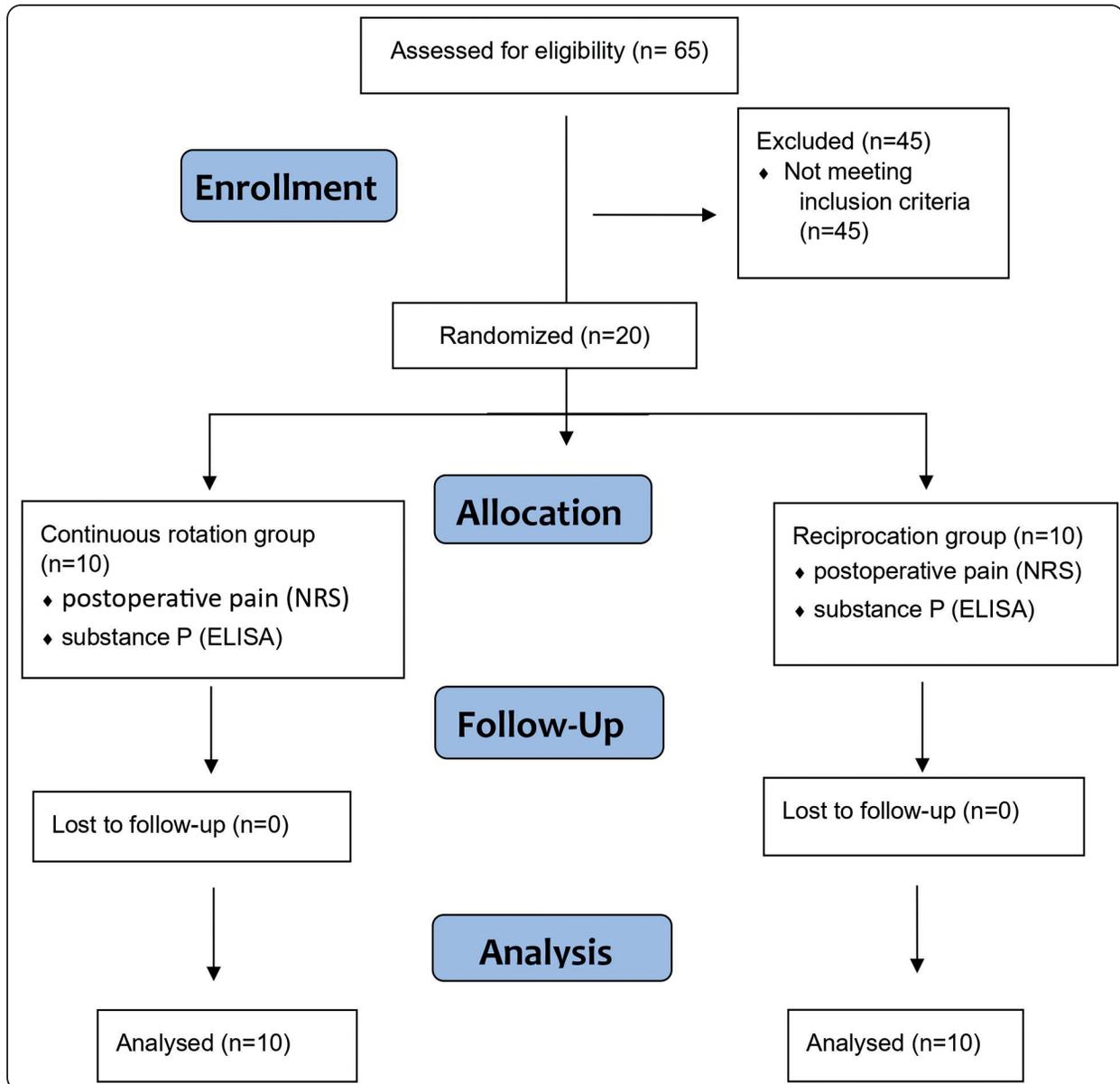


Fig. (1)

null hypothesis that there is no difference between tested groups. By adopting an alpha level of (0.05) and a beta of (0.2) i.e., power=80% and an effect size (d) of (1.25) calculated based on the results of Caviedes-Bucheli J<sup>16</sup>; the predicted sample size (n) was found to be a total of (20) cases (i.e.,10 cases per group). Sample size calculation was performed using G\*Power version 3.1.9.7 2.

### III- Eligibility criteria:

The eligibility requirements comprised individuals in good health, aged 20 to 50, who had complete root formation on their mandibular second premolars, which were single-canaled. In addition, patients had to be diagnosed with periapical symptomatic apical periodontitis and pulpal symptomatic

irreversible pulpitis, without the presence of a visible periapical radiolucent area.

Using analgesics 12 hours prior to treatment, radiographically untraceable canals, excessively curved roots, teeth with open apices, severe periodontal disease (either generalized or localized to the tooth in question), allergic reactions, systemic diseases, and the absence of bleeding in the pulp chamber upon access cavity preparation were among the exclusion criteria.

#### IV- Randomization

Ten patients each were randomly assigned to two parallel groups for comparison based on their eligibility. The EdgeOne Fire reciprocating system was used to treat RG, while the EdgeEndox7 rotary system was used to mechanically prepare CRG. The random sequence was produced by computer software, and the allocation was hidden inside folded, numbered papers that were sealed into tightly sealed envelopes with the patient's code on them.

#### V- Treatment protocol and interventions

**Diagnosis:** After gathering demographic information, an accurate diagnosis was made. When exposed to cold pulp sensibility tests (Endo- Frost, Coltene- Whaledent, Switzerland), every tooth in the study responded excessively, and extensive pulp bleeding was evident when the pulp chamber was opened. A positive response to percussion and palpation verified the periapical diagnosis.

**Access preparation:** All patients were anesthetized using 4% ARTINIBSA solution with 1:100,000 epinephrine (Inibsa, Spain) then the operative field was isolated using a rubber dam. Access cavity preparation was done under magnification.

**Working length determination:** The working length was determined using the #15 K-file (Mani, Japan) and the root ZX apex locator (J Morita,

Tokyo, Japan). Radiographic confirmation was then performed. Irrigation with 2.5% sodium hypochlorite was done after pulp extirpation and canal patency was achieved to the working length.

**Apical fluid (AF) sample collection:** A size 15.02 paper point (DiaDent Group, Seoul, Korea) was advanced 1-2 mm beyond the apex and held in place for 60 seconds in order to collect the first apical fluid sample. The paper point was then placed in an Eppendorf tube containing 1 mL of phosphate-buffered saline (PBS) (pH 7.4) and refrigerated at 10°C for further examination<sup>17</sup>.

#### Canal preparation: As per the designated group

**CRG:** The manufacturer's instructions were followed, and the canals were enlarged apically to a size of 45. At 350 RPM and 3 Ncm, a Motopex endodontic motor (Woodpecker, Guilian, China) was employed. File #25.12 was used for coronal flaring, and without missing a file, the mechanical preparation was carried out from #20.04 to #45.04 (#25.12, #20.04, #25.04, #30.04, #35.04, #40.04, and #45.04). **RG:** Using the file sequence (#25.12, #20.04, #25.04, #35.04, and #45.04), the Motopex endodontic motor (Woodpecker, Guilian, China) was set in reciprocating mode with 150 degrees Counter-Clockwise (CCW) and 30 degrees Clockwise (CW) at 300 RPM and 2 Ncm.

Conventionally, 2 mL of 2.5% NaOCl was used to irrigate each canal in between files. After applying 5 mL of 2.5% NaOCl for a minute, 5 mL of 17% EDTA (Meta Biomed, Korea) was used for a minute as a final rinse. A size 25 double-vented needle was used to deliver irrigants.

**Temporary restoration:** Canals were dried, and glass ionomer Fuji IX (Tokyo, Japan) was used to temporarily restore the access cavity.

**The second visit** was set for five days later. Following the previously mentioned steps for proper tooth isolation, the temporary filling was removed, and a second sample was taken from the

apical fluid utilizing the same protocol as the initial sample collection.

**Root canal filling:** following irrigation with 2.5% sodium hypochlorite and finishing with a last flush of 17% EDTA, interspersed with saline irrigation. Sterile paper tips were used to carefully dry the root canals. A radiograph was then taken to confirm the proper working length after a master cone, sized at #45, was carefully placed into the canals. The obturation step of the process was then carried out using warm vertical compaction with the Woodpecker obturation system Fi-P and Fi-G (Guilin Woodpecker Inc., China).

**VI- Biochemical examination**

600 milliliters of PBS were used to dilute and cut the collected paper points. After that, the materials were centrifuged for five minutes at 10,000 rpm. Substance P was counted using an enzyme-linked immunosorbent assay (ELISA) kit in accordance with the manufacturer’s instructions. With this kit, the lowest limit of SP detection was 3.9 pg mL<sup>-1</sup>. Using a microplate reader (SpectraMax Plus 384, USA), the absorbency of each sample was measured at wavelengths ranging from 420 to 450 nm. The concentration of SP in every sample was determined using a standard curve.

**VII- Statistical analysis**

Numerical data were presented as mean, standard deviation (SD), median, and interquartile range (IQR) values. They were explored for normality by checking the data distribution, and by using Shapiro-Wilk’s test. Normally distributed data (substance P) were analyzed using independent and paired t-tests for inter and intragroup comparisons, respectively. The significance level was set at p<0.05. Statistical analysis was performed with R statistical analysis software version 4.3.1 for Windows\*.

\* R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

**RESULTS**

There was no significant difference between tested groups regarding sex (P=1) and age (P=0.452) shown in Table (1) and figures (b), (c)

TABLE (1) Intergroup comparisons and summary statistics for demographic data.

| Parameter                |        | Reciprocation | Continuous rotation | P-value |
|--------------------------|--------|---------------|---------------------|---------|
| Sex<br>[n(%)]            | Male   | 6 (60.0%)     | 6 (60.0%)           | 1ns     |
|                          | Female | 4 (40.0%)     | 4 (40.0%)           |         |
| Age (Mean±SD)<br>(years) |        | 38.20±5.11    | 40.20±6.45          | 0.452ns |

\*; significant (P < 0.05) ns; non-significant (P>0.05)

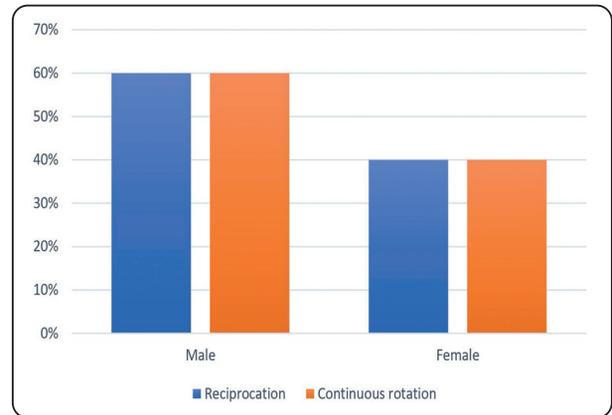


Fig. (b) Bar chart showing sex distribution.

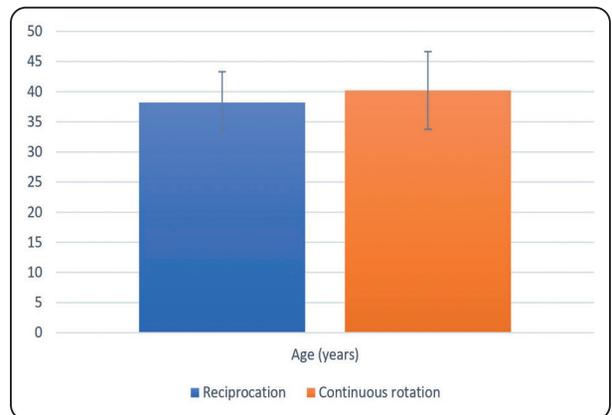


Fig. (c) Bar chart showing mean and standard deviation values for age (years).

Reciprocation (42.10±13.31) (pg/ml) had a significantly higher value in the change of SP than continuous rotation (16.19±2.20) (pg/ml) (P<0.001) explained in table (2) and figure (d).

TABLE (2) Intergroup comparisons, mean and standard deviation values of difference in substance P level (pg/ml).

| Difference in substance P level (pg/ml)<br>(mean±SD) |                     | P-value |
|--|---------------------|---------|
| Reciprocation  | Continuous rotation |         |
| 42.10±13.31  | 16.19±2.20          | <0.001* |

\*; significant (P < 0.05) ns; non-significant (P>0.05)

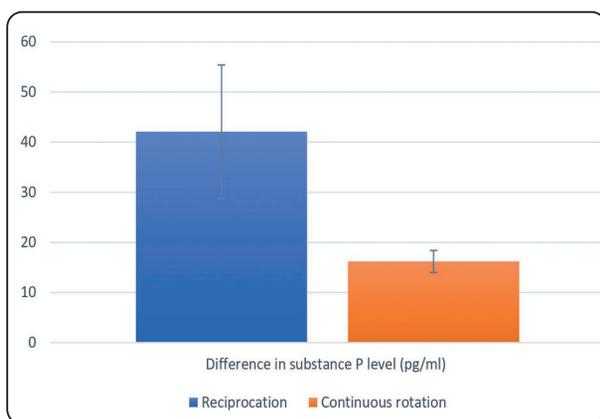


Fig. (d) Bar chart showing mean and standard deviation values for the difference in substance P level (pg/ml).

### DISCUSSION

Inflammation and tissue damage surrounding the tooth root are the hallmarks of apical periodontitis (AP), which can be brought on by trauma, endodontic treatment complications, or infections of the dental pulp<sup>18</sup>. Lipopolysaccharide (LPS), produced by infected root canal microorganisms, triggers a neutrophil and macrophage immune response. Neuropeptides like Substance P (SP) and chemokines like IL-8/CXCL8, IL-1, IL-6, and IL-17 help to promote this inflammatory response<sup>19</sup>.

The synthesis of substance P by sensory neurons and its subsequent release in response to a variety of

triggers, including heat, bradykinin, and capsaicin<sup>20</sup>, are significant factors in the pathophysiology of apical periodontitis and pain. Increased release of SP, which binds to Neurokinin 1 (NK1) receptors and triggers inflammatory and hyperalgesic responses, can also be brought on by prostaglandins, neurotrophins, and other mediators sensitizing neurons. Accordingly, SP can be a target for pain management treatments<sup>20</sup>.

Pain triggers an inflammatory response through increased extrusion of apical debris into the periapical area<sup>21,22</sup>. The kinematics of the instrumentation have a significant impact on this extrusion<sup>23</sup>.

The goal of the current randomized clinical trial (RCT) was to determine how different instrumentation kinematics affected changes in SP levels after single-rooted mandibular second premolars underwent SAP root canal therapy. The best quality of evidence is offered by RCTs since they minimize confounding variables and systematic error (bias)<sup>24</sup>. As a result, they offer the most precise and reliable information regarding an intervention's effectiveness.

In symptomatic irreversible pulpitis, hyperalgesia and allodynia (in peripheral and central pathways) continue even after root canal therapy<sup>25</sup>. Therefore, in order to guarantee the presence of inflammation in and around the root, single-root mandibular second premolars with irreversible pulpitis and symptomatic apical periodontitis were selected for inclusion.

Patients were only eligible to participate in the study if they had not taken any painkillers 12 hours prior to the visit in order to eliminate the impact of pre-treatment analgesics on pain analysis<sup>26</sup>.

In order to guarantee the vitality of the pulp, methods such as applying cold to cause pain and allowing pulp bleeding during access cavity preparation were employed<sup>27</sup>. Pain during percussion was used to diagnose apical periodontitis<sup>28</sup>.

Contrary to other research<sup>29</sup>, some studies found no statistically significant difference in postoperative pain severity between men and women<sup>30,31</sup>. When participant age and sex were taken into account, the current study did not find any significant differences between the two groups.

To guarantee that the rotary Ni-Ti systems in this study experienced a comparable heat treatment procedure which is the FireWire™ treatment, systems from the same manufacturer were selected. This involves a combination of cryogenic applications and heat treatment to reduce restoring force and improve file flexibility, according to claims made by the manufacturer<sup>8,32</sup>. The EdgeEndo x7 files were used in a continuous rotation motion and the EdgeOne Fire files were used in a reciprocating motion with parameters set at 150 degrees CCW and 30 degrees CW. Yet, they differed in their kinematics where the EdgeEndo x7 files were employed in a continuous rotation motion, while the EdgeOne Fire files were utilized in a reciprocating motion<sup>6,9</sup>.

Because paper points are thought to be the best method for reaching the highest fluid levels at the apical section, even when working with small amounts of exudates, they were utilized to analyze the levels of SP in the apical fluid<sup>33</sup>. Because 7.4 PH phosphate buffer saline has non-toxic qualities and can stop sample cell rupture from osmosis, it was used to hold all paper points containing apical fluid samples. This ensured the sample's stability until additional testing<sup>34</sup>.

The use of rotary instruments in canal preparation is linked to a lower incidence of inflammation than reciprocating instruments because of the decreased debris extrusion caused by the continuous rotation, which reduces irritation and minimizes inflammation<sup>35</sup>. The reciprocating motion involves an initial rotation in a counterclockwise direction, which allows the instrument to penetrate and cut the dentin. Thereafter, it follows a rotation in the opposite direction, which makes the flutes unable to

remove the debris but rather push them apically<sup>36</sup>.

Additionally, the EdgeOne Fire sequence's lack of size #30 and #40 reciprocating files results in higher cutting pressure on the larger reciprocating files, which increases debris accumulation and this falls in line with Nevares et al.'s findings<sup>37</sup>.

The full sequence rotary files in this study effectively reduced debris buildup even though the irrigation volumes used for the rotary and reciprocating groups were equal. This was due to the higher frequency of irrigation cycles which in return lessened the possibility of apical extrusion<sup>38</sup>.

These results also align with earlier studies that examined the apically extruded debris with reciprocal and continuous rotational files, concluding that reciprocal motion leads to a higher apical extrusion of debris than continuous rotary motion<sup>39</sup>.

While some found no discernible difference<sup>40</sup>, others reported lower levels of inflammation with reciprocation compared to continuous rotation<sup>22</sup>.

Various file designs, variations in the experimental setup, and the subjectivity of pain as reported by various patients could all be contributing factors to this discrepancy.

Based on the information that is currently available, no prior research has examined the impact of reciprocation kinematics and continuous rotation on SP simultaneously. Caviedes-Bucheli et al.<sup>16</sup> looked into how different rotary instrumentation systems, such as ProTaper Universal, RaCe, and Mtwo, affected substance P levels. They found that Mtwo had the lowest substance P expression, most likely because of its EdgeEndo x7-like design, which makes debris removal more effective.

## CONCLUSION

Continuous rotation motion is accompanied by lower levels of substance P when compared to reciprocation motion in patients with symptomatic apical periodontitis.

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