Outcome of endodontic microsurgery performed by postgraduate students: A retrospective study

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Introduction

Endodontic surgery is commonly performed to treat patients who exhibit signs and symptoms of periapical disease resulting from unsuccessful non-surgical endodontic treatment. The main goal of this procedure is to prevent bacterial invasion and associated endotoxin by-products from entering the root-canal space and periradicular tissues, and to remove persisting lesions (1,2). An absence of symptoms during the clinical examination (2) and total elimination of periapical radiolucency are considered signs of successful surgery (3,4).

Purpose: The aim of this study was to survey the outcomes of endodontic microsurgery performed by endodontic postgraduate residents.

Methods: Clinical and radiographic data of 70 patients who underwent endodontic microsurgery between January 2015 and March 2022 were collected through the clinical electronic system of the university clinics. Each patient was contacted for a follow-up appointment. Clinical and radiographic examinations were performed for each patient, and the success rate and tooth survival were analyzed.

Results: Sixteen patients (24 teeth), mostly female, attended the follow-up appointments with an average follow-up period of 18 months. Most of those who did not show up had no problem and some did not respond, while the rest changed their addresses and others were not interested in attending. Persistent periapical infection followed by overfilling was the most common reason for periapical surgery. All teeth (100%) survived. Out of 18 maxillary teeth, complete healing was observed in 44.4% and incomplete healing in 44.4%, whereas only 33.3% of six mandibular teeth displayed complete healing and 66.7% had incomplete healing. The combined success of complete and incomplete healing was 91.66%. However, the distribution of the outcome levels within each location, maxillary and mandibular, showed no significant difference (p > 0.05).

Conclusion: A predictable high success rate of endodontic microsurgery treatment performed by endodontic postgraduate residents of complete and incomplete healing was achieved.

Keywords: Endodontic microsurgery; follow-up; outcome; postgraduate residents; retrospective study.
ably with the introduction of microsurgery, with success rates of over 90% (6-8). The improvement is attributed to the use of magnification, ultrasonic surgical tips, and biocompatible retrograde filling materials such as Biodentine and mineral trioxide aggregate (MTA) (6,9-11).

Surgical-site healing is usually evaluated at 1 year after surgery (2), although small defects in a periapical lesion may heal within a few months (12). Outcome studies with long-term follow-ups have demonstrated that initial success rates remain high and fairly constant over time (6-8,11,13-15).

A limited number of studies have investigated endodontic microsurgery performed by endodontic postgraduate residents (6,7). In addition, two studies evaluated the cases of both endodontic postgraduate residents and faculty (11,16). Currently, no data have been published regarding the outcomes of traditional endodontic surgical or microsurgical cases performed by postgraduate residents of a Saudi University. The aim of this study was to survey the outcome of endodontic microsurgery performed by endodontic postgraduate residents.

Materials and Methods

This study was approved by the Scientific Research and Ethics Committee of Riyadh Elm University (IRB approval number: FPGRP2021670723848). A consent form explaining the importance of the follow-up visit and the examination steps was signed by each patient.

Sample size

The dental records of 70 healthy patients who had endodontic microsurgery treatment performed at the postgraduate clinics of Riyadh Elm University Hospital by endodontic postgraduate residents between January 2015 and March 2022 were reviewed. The coded computerized program of the clinical-treatment procedures used at Riyadh Elm University Hospital facilitated the tracking of cases.

The following data were collected: age, sex, medical status, tooth type, clinical and radiographic diagnosis of the involved teeth, the reason for endodontic microsurgery treatment, the type of treatment performed, and the presence of coronal restoration. Eligibility for the study was assessed based on the following inclusion criteria:

1. Complete endodontic microsurgery and restorative treatment record of healthy patients attending the recall visit.
3. The treatment is performed at least 6 months before the recall visit by endodontic postgraduate residents.

Cases that did not meet the inclusion criteria were excluded.

Surgical procedure

The endodontic microsurgery treatment conducted by endodontic postgraduate residents at university clinics followed the recommendations of the American Association of Endodontics [Contemporary Endodontic Microsurgery: Procedural Advancements and Treatment Planning Considerations (Fall 2010), available at: https://www.aae.org/specialty/newsletter/contemporary-endodontic-microsurgery (accessed on April 15, 2023)]. Clinical and radiographic examinations were usually performed, and individual cases were discussed with the supervising teaching staff. The treatment plan and the surgical procedure were explained by the endodontic resident to each patient before starting the surgery, and a consent form was obtained. The procedure was carried out with the aid of a dental operating microscope (Global Surgical Corporation, St. Louis, MO, USA). After flap reflection, osteotomy was performed using a round bur attached to a high-speed handpiece to gain access to the root apex and the periradicular lesion. Subsequently, the apical third of the root apex was resected (≤ 3 mm) with minimal or no bevelling. The lesion was curetted if present, and hemostasis was achieved with racellet epinephrine pellets (Pascal Co. Inc., Bellevue, WA, USA). To identify any isthmuses, accessory canals, or cracks, the resected roots were stained with methylene blue dye and scrutinized with a micro-mirror. Retro-apical preparation was performed (≥ 3 mm) with KiS ultrasonic tips (Kavo Kerr Dental, Joinville-le-Pont, France). The surgical site was flushed with a normal saline solution and dried with surgical suction. MTA (ProRoot, Dentsply Tulsa, OK, USA) was used as a retro-fill material and plugged with angled micro-pluggers. A periapical radiograph was taken, then a Vicryl suture was used to secure the flap. The patients were prescribed analgesics, antibiotics, and a 0.2% chlorhexidine mouth rinse twice daily for 1 week. All procedures were performed under the close supervision of endodontic staff. Sutures were removed at 3–5 days after surgery in most cases.

Follow-up appointment procedure

Each patient was contacted for a follow-up appointment. Those who did not respond were contacted again. Each patient was appointed 20 min for clinical and radiographic examination. The clinical examination was performed by the second co-author LA and periapical radiographs were taken by a well-trained technician. An extension-cone-parallelizing dental-X-ray film-positioning device was used to increase the dimensional accuracy of the X-ray imaging.
Clinical and radiographic examination

A routine clinical examination was conducted for treated teeth to assess the treatment area and periodontal condition. The status of the periapical area in recalled radiographs was compared to radiographs taken at the end of the surgical procedure. The criteria set by Rud et al. (3) and Molven et al. (4,17) were used to evaluate the periapical radiographs. The clinical and radiographic findings were evaluated on the basis of survival and success. Survival was defined as the tooth still being present in the arch after treatment and at the time of the recall visit. Clinical success was defined as a surviving tooth without clinical symptoms and not requiring additional treatment intervention during the evaluation period. A multi-rooted tooth was evaluated as one unit; a tooth was considered functional when an absence of symptoms was noted, regardless of the status of the periapical area. Any complication such as recurrent caries, root resorption, and/or fracture was recorded. Cases scored as complete or incomplete healing were pooled together and considered successful (17). Any indication of unsatisfactory healing at the 1-year follow-up was considered a failure. One form was used for each tooth.

Data evaluation

Two qualified endodontists, blind to the study, reviewed the results of the clinical and radiographic examinations and assessed the outcome of each case independently. Each tooth was evaluated in its entirety for preoperative, intra-operative, and postoperative factors. Assessment of all radiographs was carried out under standardized viewing conditions using a computer with a high-quality screen. In the case of disagreement, both endodontists discussed the case until consensus was reached.

Statistical analysis

Data were coded and entered into a database for statistical analysis. The collected data were analyzed using SPSS ver. 26.0 for Windows statistical software (IBM Corp., Armonk, NY, USA). The chi-squared test was used to study the associations between categorical variables. A proportional t-test was used to compare males to females, and inter-examiner reliability was measured using Cohen’s kappa coefficient ($\kappa$). All tests were interpreted at the 5% significance level ($p \leq 0.05$).

Results

In total, 24 microsurgically treated teeth of 16 healthy patients who attended the follow-up visit were evaluated. Root canal retreatment was carried out on 12 teeth before the surgery and 5 teeth had post. Five teeth had well condensed root canal filling and the apical third of 2 teeth were calcified. All evaluated teeth had sufficient coronal restoration.

The follow-up period was extended from 6 months to 3 years, with an average of 21.9 months. Fifty-four patients (77.14%) were categorized as lost, as they did not attend the follow-up visit. The reasons for the patients not attending the follow-up examinations were as follows: 19 (had no problem), 12 (did not respond), 5 (moved outside Riyadh city), 6 (failed to attend), and 4 (provided the wrong phone number). In addition, 8 patients answered that they were not interested in being followed-up after the procedure.

The kappa scores were $\kappa = 0.88$ for inter-examiner agreement and $\kappa = 0.64$ for intra-examiner agreement.

Most of the patients were women (56.3%) aged 20–39 years. The patients’ sex was not a significant factor ($p = 0.617$). Similarly, the multinomial proportional test showed no significant difference between age levels ($p = 0.209$).

Out of 18 maxillary teeth, complete healing was observed in 44.4% and failure in 11.2%. In six mandibular teeth, only 33.3% of teeth showed complete healing. The combined success of complete and incomplete healing was 91.66%. The distribution of outcome levels for each location, maxillary and mandibular, showed no significant difference ($p > 0.05$), but the overall or total of maxillary

| Table 1. Distribution of outcome of Endodontic microsurgery in relation to the location of tooth |
|----------------|----------------|----------------|----------------|----------------|----------------|
| Tooth location | Complete healing | Incomplete healing | Failed | Total | Ch-sq p-value | Multinomial p-value |
| Maxillary      | 8 (44.4%)        | 8 (44.4%)        | 2 (11.2%) | 18 (100%) | 0.410          | 0.527            |
| Mandibular     | 2 (33.3%)        | 4 (66.7%)        | 0 (0%)   | 6 (100%) | 0.410          | 0.329            |
| Total          | 10 (41.7%)       | 12 (50.0%)       | 2 (8.3%) | 24 (100%) | 0.410          | 0.030            |
and mandibular locations differed significantly ($p = 0.030$) (Table 1). The numbers of teeth treated were higher in females than in males (Table 2). The multinomial test showed the distribution of outcome levels for each location, with no significant difference within female/male ($p > 0.05$). Overall, the total values for male and female differed significantly ($p = 0.030$).

More teeth were located in the maxillary anterior region (Table 3). The multinomial test showed that the distribution of outcome levels for each location was not significantly different ($p > 0.05$). Overall, the total values for maxillary and mandibular regions differed significantly ($p = 0.030$).

The analysis of Endodontic microsurgery treatment outcomes according to gender (Table 2) showed that the numbers of teeth treated were higher in females than in males ($p = 0.030$). The multinomial test showed the distribution of outcome levels for each location, with no significant difference within female/male ($p > 0.05$). Overall, the total values for male and female differed significantly ($p = 0.030$).

Table 2. Analysis of Endodontic microsurgery treatment outcomes according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of teeth</th>
<th>Complete healing</th>
<th>Incomplete healing</th>
<th>Failed</th>
<th>Ch-sq p-value</th>
<th>Multinomial p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>0</td>
<td>0.537</td>
<td>0.400</td>
</tr>
<tr>
<td></td>
<td>(40%)</td>
<td>(60%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>14</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(42.85%)</td>
<td>(42.85%)</td>
<td>(14.28%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>10</td>
<td>12</td>
<td>2</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(41.66%)</td>
<td>(50%)</td>
<td>(8.33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Analysis of Endodontic microsurgery treatment outcomes according to tooth type (jaw)

<table>
<thead>
<tr>
<th>Jaw</th>
<th>Tooth type</th>
<th>Number of teeth</th>
<th>Complete healing</th>
<th>Incomplete healing</th>
<th>Failed</th>
<th>Multinomial p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxillary</td>
<td>Anterior</td>
<td>17</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(41.17%)</td>
<td>(47.05%)</td>
<td>(11.76%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premolars</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molars</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>8</td>
<td>8</td>
<td>2</td>
<td></td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>(44.44%)</td>
<td>(44.44%)</td>
<td>(11.11%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mandibular</td>
<td>Anterior</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Premolars</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molars</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(50%)</td>
<td>(50%)</td>
<td>(50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td></td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>(33.33%)</td>
<td>(66.66%)</td>
<td>(100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Description and analysis with percent frequencies of Endodontic microsurgery outcomes in relation to patient/procedure complications. A Multinomial test.

<table>
<thead>
<tr>
<th>Patient/Procedure complication</th>
<th>Number of teeth</th>
<th>Complete healing</th>
<th>Incomplete healing</th>
<th>Failed</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent periapical infection</td>
<td>17</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td>0.113</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(35.29%)</td>
<td>(52.94%)</td>
<td>(11.76%)</td>
<td></td>
</tr>
<tr>
<td>Overfilling</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0.317</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(75%)</td>
<td>(25%)</td>
<td>(50%)</td>
<td></td>
</tr>
<tr>
<td>Underfilling (short)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(0%)</td>
<td>(0%)</td>
<td></td>
</tr>
<tr>
<td>Cannot do RCT retreatment (Calcified)</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(100%)</td>
<td>(100%)</td>
<td>(100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>10</td>
<td>12</td>
<td>2</td>
<td>0.030</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(41.66%)</td>
<td>(50%)</td>
<td>(8.33%)</td>
<td></td>
</tr>
</tbody>
</table>
tion of outcome levels within anterior teeth in the maxillary arch did not differ significantly \( (p > 0.05) \); however, overall \( p = 0.035 \). A few mandibular teeth were also evaluated (Table 3); the distribution of outcome levels within molar teeth and overall showed no significant differences \( (p > 0.05) \).

Persistent periapical infection followed by overfilling was the most common reason for periapical surgery (Table 4). Among the 17 surgical cases due to persistent periapical infection, six teeth had healed completely and two had failed to heal (Fig. 1-4). Similarly, three teeth exhibiting overfilling healed completely and one tooth healed incompletely. The distribution of outcome levels within persistent periapical infection and overfilling was not significant \( (p > 0.05) \), but overall \( p = 0.03 \).

**Discussion**

This study aimed to evaluate the outcomes of microsurgical endodontic treatment performed by endodontic postgraduate residents at Riyadh Elm University Hospital. Few studies evaluating the treatment outcomes of postgraduate cases have been reported \((7,11,16)\); but among the few, high success rates were indicated. These findings are consistent with those of the present study. The kappa scores of the present study were considered very good following the criteria of Landis and Koch \((18)\), were similar to those of Eskandar et al. \((16)\), and were better than those of Azim et al. \((11)\), who reported moderate reliability \((0.61)\).

The surgical procedure was carried out with the aid of a dental operating microscope. Contradictory findings on the impact of magnification and illumination on root-end-surgery outcomes have been reported. In one study, a significantly higher success rate was reported with microscope use compared to without its use, as specified by Tsesis et al. \((9)\). Another study found microscope use to be a non-significant prognostic factor \((19)\). We consider that use of magnification is mandatory as it provides the trainee resident with a clear view of the surgical site and the potential for improved detection of problems.

Periapical radiographs were used to assess healing of the periapical area of the treated teeth in this study, following the criteria recommended by Rud et al. \((3)\) and Molven et al. \((4)\). This is the method most commonly used to assess the outcomes of endodontic traditional and microsurgery treatments and has been used in many studies \((7,11,13,14,16,20)\). The American Association of Endodontics/American Academy of Oral and Maxillofacial Radiology \((21)\) reported that cone-beam computed tomography (CBCT) is a good tool for outcome evaluation during preoperative assessment of endodontic cases.
Several studies have used CBCT to evaluate endodontic-surgery outcomes (6,11,16,22,23). Curtis et al. (22) found CBCT to be more sensitive and accurate than periapical radiographs in assessing periapical healing. In contrast, Azim et al. (11) reported slight differences between CBCT and periapical radiographs; they were in favor of periapical radiographs, although the CBCT evaluations were more consistent. In recent studies, Eskandar et al. (16) reported some agreement between periapical radiograph and CBCT outcomes, albeit showing a significant difference (p = 0.029). In contrast, Gurusamy et al. (23) found that CBCT did not exhibit any additional advantage over periapical radiography when assessing healing outcomes of endodontic microsurgery.

The number of teeth evaluated in this study was small, but similar to the numbers evaluated in previous studies by Pacora et al. (24) (sample size = 10), Taschieri et al. (25) (sample size = 21), and Christiansen et al. (26) (sample size = 25). Although many patients did not attend their follow-up visit, the evaluated cases seemed precise, as all of the teeth survived during the follow-up period. In fact, exclusion of these cases would have affected the final outcome. Systematic reviews by Torabinejad et al. (27) and Chércoles-Ruiz et al. (28) reported high survival rates of teeth treated by microsurgery, consistent with the results of the present study. However, owing to the small sample size, the limited statistical power of the study, and the small number of failures recorded, these results must be considered as strictly pertinent to the observed sample and cannot be generalized.

The observation periods of the cases included in this study were 6–36 months; the majority were followed up after 24 months. Although a short follow-up period may lead to unfair bias in results and recurrence of apical periodontitis cannot be captured (10), several reports have shown that the outcomes of cases determined to be a success or failure after 6–12 months usually demonstrated the same healing pattern after longer follow-up periods (3,29). Molven et al. (17) considered cases of incomplete healing at 1 year postoperatively as being successful, as these cases had healed with scar tissue. In addition, prolonged observation of incompletely healed cases showed that they often ultimately resolved as either completely healed or incompletely healed with scar tissue (30–32). In the present study we combined the incomplete and complete-healing cases into one group and classified them as being a success, similar to the studies by Tsesis et al. (9), Huang et al. (32), Molven et al. (17) and Tortorici et al. (33). On this basis, the success percentage in the present study was 91.66%. This is consistent with the findings of Tsesis et al. (9) and Song et al. (34).

Several outcome-predictor factors (e.g., age, sex, lesion size, tooth type and location, and retro-filling material) have been investigated. In this study, no significant difference was found between men and women. Similar findings were reported by Azim et al. (11), Tsesis et al. (30), Huang et al. (32), Zuolo et al. (35), Rahbaran et al. (36), von Arx et al. (37), and Wang et al. (38). Age was not considered a prediction factor in most studies (7,16,30,35,36,38). However, Barone et al. (39) reported that older patients (> 45 years old) exhibited better healing compared to younger ones, whereas Von Arx et al. (31) reported the opposite trend, although the difference was not significant (9). No significant difference in treatment results related to the tooth type or location was found, similar to previous reports (30,35). However, owing to the small sample size, the small number of failures recorded, and the limited statistical power of the study, these results must be considered as strictly pertinent to the observed sample and cannot be generalized.

Persistent periapical infection, the main causative factor of surgery in this study, is associated with intra- and extra-radicular infection, the foreign-body reaction, cyst formation, and fibrous scar tissue healing (40,41). To obtain better outcomes, ultrasonic root-end preparation of the root canal and isthmuses and removal of discolored dentin that may harbor necrotic tissue and bacteria and associated by-products are important. In this study, the success percentage of cases with persistent periapical infection was 88.23% when complete and incomplete healings were combined. No histological examination to determine the type of periapical lesion removed was conducted for the evaluated cases.

This study evaluated levels of preoperative root filling length, and found this factor to be a major predictor of outcomes, as reported previously (38). All of the treated cases in the present study were referred from outside clinics; therefore, the root-canal filling material was not known. Studies have shown that some types of sealer components, such as eugenol and paraformaldehyde, are toxic and may inhibit conduction of the action potential of nerves to varying degrees and delay healing of surrounding periodontal and osseous cells (42,43). In the present study, treated teeth with long or short fillings had a combined healing rate of 100%. Healing in teeth with short root fillings can be enabled only by surgical resection of
the infected portion of the root (38), whereas healing in teeth with long root fillings can be induced by surgical removal of extruded filling material or canal debris colonized by microorganisms (44,45). In general, healing of a periapical lesion usually occurs if it is adequately managed (46,47).

A previous study concluded that periapical bone healing occurred independent of the placement of a root-end filling (48). This may be true if the root canal is well cleaned and filled. An MTA root-end filling was used in all cases to ensure maximum security at the apical area. In a randomized clinical trial, Christiansen et al. (26) demonstrated the importance of placing a root-end filling after root-end resection. Furthermore, they showed that root-end resection, followed by MTA root-end filling, was successful in patients with periapical disease. Similar findings have been reported in the present study and by Chan et al. (7), Azim et al. (11), Chong et al. (13), Eskandar et al. (16), Lindboom et al. (20), and Christiansen et al. (26).

None of the cases evaluated was treated because of failure of previous apical surgery. However, based on previous studies, success rates of repeated surgery compared to first-time surgery may or may not differ significantly (31,38,49).

The small number of cases, resulting from the low percentage of patients who attended their follow-up visit, is considered a limitation of this study. In addition, external validation was limited, as the cases were retrieved from a single center (Riyadh Elm University Dental Hospital). Additional studies with a larger sample size are required to improve the power of the statistical analysis.

**Conclusion**

The success rate of endodontic microsurgery treatment performed by endodontic postgraduate residents at Riyadh Elm University Hospital was high. Persistent periapical infection was the most common reason for periapical surgery. Despite the small number of cases evaluated, we consider that this study provides both important information and valuable insights for endodontic practices, and a basis for future studies.


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**Source of Funding:** None declared.

**Conflict of Interest:** None declared.

**Ethical Approval:** The study protocol was approved by the Riyadh Elm University Ethics Committee (date: 30.03.2023, protocol no: FPGRP2021670723848).

**Informed consent:** Written informed consent was obtained from patients who participated in this study.

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