

Original Article

Treatment outcomes for surgical and non-surgical endodontic retreatment of teeth with apical periodontitis

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Abstract

This study was conducted to investigate the treatment outcomes of root canal treated teeth with apical periodontitis which were treated either non-surgically or by means of endodontic microsurgery and assessment of factors such as presence/absence of intra-radicular posts, clinical and demographic factors on outcome of treatment modality adopted. The data comprising of clinical and radiographic record of 398 patients with previously root filled teeth and periapical periodontitis with follow-up period of more than 11 months were included in the study. Non-Surgical cases were evaluated using the peri-apical index (PAI) sub-divided into 3 groups i.e., success, uncertain and failure at control. Similarly, surgical cases were assessed using Molven/Rud's criteria distributed into 3 sub-groups i.e., success, uncertain and failure at control. The results of present study showed that the patient's underage of 50 years (88.67%) and above 50 years (76.96%) preferred NSRT over EMS (11.3% & 23.04%) whereas, the percentage of EMS was greater in patients >50 years (23.04) compared to <50 years (11.33). The success rate of surgical endodontic procedures was significantly higher than that of non-surgical treatment ($p = .035$). Conversely, the subgroups of teeth that had posts tended to do somewhat better following non-surgical therapy as opposed to surgical treatment ($p = 0.111$). There was a significant difference ($p = .018$) favouring surgical treatment for subgroups without a post. These results concluded that compared to non-surgical retreatments, this study indicated that surgical retreatments had a better success rate. On the other hand, non-surgical retreatments, as opposed to surgical retreatments, typically yielded superior results for teeth with intra-radicular posts.

KEYWORDS: Non-Surgical Retreatment [NSRT], Endodontic Microsurgery [EMS], Periapical Periodontitis, Peri-apical Index [PAI], Root Filled Teeth.

Introduction:

The conservative inclination toward root canal therapy rather than tooth extraction has resulted in a substantial increase in the number individuals seeking endodontic treatment in recent years (1). The purpose of root canal therapy is to eliminate necrotic tissue, clean and disinfect the root canal system to lower the quantity of microorganisms, and seal the system to stop recontamination. For endodontic primary treatment, success rates as high as 97% have been documented (2); nevertheless, failure may arise following the treatment. Moreover, a foreign body reaction could be triggered by microorganisms and root filling material contaminating the peri radicular tissues, which would hinder tissue repair.

One of the frequent clinical disorders affecting the peri-radicular tissues is periapical lesions (3). The beginning and development of periapical lesions are mostly caused by microbial infiltration and subsequent infection of a root's canal systems (4). Radicular cysts, oral granulomas, and abscesses are the three main classifications for parietal lesions (5, 6). Cyst incidence ranges from 6% to 55% of all periapical lesions (7). Moreover, the prevalence of abscesses ranges from 28.7% to 70.07%, and that of granulomas from 9.3% to 87.1% (8). More substantial lesions are probably radicular cysts, based on clinical data. Nevertheless, granulomas may appear to be some of these large tumors (9). The primary goal of every endodontic procedure, particularly the cleaning and shaping steps, is to eradicate infectious bacteria and necrotic tissue (10). Both large periapical lesions and apical true cysts are inflammatory in nature, and nonsurgical treatment is the best course of action in the beginning (9). A surgical approach should only be considered in cases of persistent intra- or extra-radicular infections and periapical disease that

does not improve with nonsurgical endodontic therapy methods (11). Numerous international cross-sectional studies have reported that more than 30% of people with root-filled teeth may have apical periodontitis and other post-treatment peri-radicular disorders (12-14). These findings point to a significant need for this condition's treatment. In order to make an informed decision regarding non-surgical (orthograde) re-treatment, surgical (retrograde) operation, or tooth extraction, periapical condition evaluation is a crucial component of re-treatment planning (1). Studies conducted on general practitioners and endodontists have revealed significant differences in the decision-making process when deciding between orthograde re-treatment and surgery (15-17). This heterogeneity may be largely attributed to individual variations in the evaluation of the severity of the disease as well as the imprecision of data about endodontic re-treatment outcomes seen in the literature (18-20). To the best of author's information, there's very limited data available on comprehensive research on treatment outcomes for surgical and non-surgical endodontic retreatment of teeth with apical periodontitis with a sizable sample size in the south Asian population of Pakistan.

Materials & Methods

Data collection:

The patient's database was collected by authors of this study from various hospitals and clinics of Pakistan where endodontic retreatment was either performed by consultants having specialization in restorative dentistry/endodontics or the postgraduates in their final year of specialization training in operative dentistry & endodontics where NSRT and EMS were performed under strict

supervision of restorative/endodontics consultant.

This was an observational retrospective cohort study comprising of total sample size of 875 patient's records with minimum follow-up period of 12 months. The data collected comprised of the patient's records encompassing clinical and radiographic data of past 5 years from 2018 to present [$>11- > 36$ months recall period] recovered from the electronic records who visited the operative and endodontics department, patients who underwent orthodontic treatment and developed apical periodontitis in root canal treated teeth. The samples were selected using non-probability purposive sampling. Of the 875 records, a total of 398 cases and records (295 non-surgical retreatments [NSRT] and 103 endodontic microsurgery cases [EMS]) were confirmed to be included in this study.

Ethical approval was obtained from Institutional Review Board (IRB) and the data from various hospitals and clinics was obtained with the permission and consent of consultant restorative dentists/endodontists.

Selection of cases:

Of the 875 teeth, only 398 cases—295 non-surgical retreatments and 103 endodontic microsurgery cases—met the inclusion criteria. Cases with radiographic symptoms of uncertain apical periodontitis (pre-operative periapical index [PAI]) score 1 or 2 were excluded, as were cases with fewer than 11 months of follow-up data (21). In situations of retreatment and numerous teeth exhibiting symptoms of apical periodontitis, the most distal tooth was the only tooth included in the study.

For every case, the following data was gathered: the patient's age and gender; the treated tooth's PAI score; whether or not a post was present; the type of therapy (endodontic microsurgery [EMS] vs. non-surgical retreatment [NSRT]); and the duration from treatment to control. If a non-surgical case was later treated surgically, only the surgical case was considered. From 2018 to present, treatment methods for NSRT and EMS remained constant with few changes, adhering to the European Society of Endodontology Guidelines [ESE] (22). Only four cases involved Intermediate restorative materials, while MTA material was employed in the majority of retrograde fillings.

Radiographic evaluation:

The PAI scoring system was used to rescore control radiographs and pre-operative imaging. A follow-up radiograph was identified and scored on the most recent date that was noted. Principle investigator did all of the scoring initially followed by co-investigators to increase the reliability of the study. The Digora system (DIGORA Accessory intraoral imaging plates, Cary) was used to acquire all digital x-rays, which were then analyzed on a screen. In order to calculate for PAI, 100 periapical radiographs were selected with the scores (Cohen's Kappa = 0.81-1.00) was used. The radiographs were assessed twice, with gap of few months with the purpose of evaluating the surgical results, using the standards established by Molven et al. (23) and Rud et al. (24). An endodontist with experience of more than 30 years were recruited for a final evaluation in those 08 cases where there was uncertainty. A final agreement was gained following a meeting with a second endodontist who inspected the microphotographs

once again and possessed the same level of knowledge spanning over 30 years.

Assessment tools:

- A. **NSRT:** Peri-apical index [PAI] was used to monitor the outcome of non-surgical retreatment cases divided to 3 categories i.e.; *Clinical success, uncertainty and failure*. The scores adjusted to reflect clinical success (PAI1 and PAI2 at follow-up), uncertainty (PAI3 at follow-up for initial PAI45), and failure (persisting PAI3 and anyPAI4 or PAI5 at follow-up). Failure was also documented if a tooth had been extracted or there was evidence of a sinus tract involving the periapical area at the time of recall.
- B. **EMS:** Surgical cases were divided into three categories: *Clinical success, uncertain, and failure*. Cases which required extraction and sinus tract formation as in non-surgical instances were also included and considered as failure.

Statistical analysis

The chi-square test was performed to compare treatment outcomes across tooth groups. Logistic regression analysis of outcome was conducted separately on surgical and non-surgical patients, with unclear cases grouped as "failures" for analytic purposes. All analyses were performed using Statistical package for Social Sciences (SPSS) Version 26 to compile and analyze data. Since this was an observational study, no level of significance was established, and the data were presented as associated p values only.

Results

- a. **General Characterization of the Data:** Following the inclusion/exclusion procedures, 103 cases were surgically treated and 351 cases were non-surgically treated (Table 1). Forty cases with a pre-operative post were in each group.

Table 1: Distribution of Patient's sex, age and pre-operative PAI Scores for the treatment of two treatment groups (and time to recall [<18m/18-36m/.36m])

Treatment	NSRT	EMS	Total (n)	Chi Squared
Female	75.89	24.11	190	0.880
Male	76.29	23.01	208	
Age <50	88.67	11.33	211	<0.001
Age >50	76.96	23.04	187	

Recall 12 months to <18 months	82.42	17.58	137	0.029
Recall >20 months	75.11	24.89	67	
Recall 18-36 months	77.20	22.80	92	
Recall >36 months	75.51	24.49	43	
PAI start 3	77.15	22.85	187	0.969
PAI start 4	77	23	125	
PAI start 5	76.80	23.20	86	
Total	74.51	25.49	398	

a. Groups of Teeth and Groups for Treatment:

Patients' genders did not differ significantly in the distribution of tooth groups; however, younger patients received treatment for comparatively more front teeth and lower molars than older patients, who were more likely to have premolars and maxillary molars. Maxillary anterior teeth received more EMS and mandibular molars following maxillary molars received highest

NSRT.

In both treatment groups, the distribution of male and female individuals was almost equal, as was the distribution of pre-operative PAI scores. Patients scheduled for endodontic microsurgery exhibited a significant age difference as compared to non-surgical retreatment: a higher proportion of elderly patients had surgery ($p < 0.001$, Table 1). Premolars and maxillary front teeth were overrepresented in surgical cases ($p < 0.001$, Table 2).

Table 2: Distribution of teeth over treatment groups (Percent)

Jaw	Maxilla	Mandible
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Tooth Group	Anterior	Premolar	Molar	Anterior	premolar	Molar	Total, n
NSRT	7.45	13.22	27.79	4.74	14.23	32.54	295
EMS	23.30	23.30	22.33	9.70	11.65	6.7	103
Chi-Squared	P < 0.001						

b. Posts

Posts were common in premolars (mandibular; 34.23% and maxillary 28.22%), and when present maxillary premolars and anterior teeth were preferentially treated with surgery ($p = 0.048$ Table 4). Conversely, teeth without posts were relatively more often retreated non-surgically ($p < 0.001$, Table 4).

Table 3: Distribution of teeth with or without post over tooth groups (Percent)

Jaw	Maxilla			Mandible			
Tooth Group	Anterior	Premolar	Molar	Anterior	premolar	Molar	Total, n
Post	5.74	28.22	17.79	3.54	34.23	10.48	80
No post	23.30	23.30	22.33	9.70	11.65	6.7	318
Chi-Squared	P < 0.048						

Post- Presence of intra-radicular post at treatment initiation; No post-presence of intra-radicular post at treatment initiation.

Table 4: Distribution of teeth with or without post over treatment groups (Percent)

Jaw	Maxilla			Mandible		
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Tooth Group	Anterior	Premolar	Molar	Anterior	premolar	Molar	Total, <i>n</i>
Post NSRT	5.74	28.22	17.79	3.54	34.23	10.48	54
Post EMS	23.30	23.30	22.33	9.70	11.65	6.7	26
Total post	10.67	26.00	22.76	3.21	12.55	26	80
No Post NSRT	10.53	18.20	37.98	5.60	4.30	27.33	240
No Post EMS	30.00	21.98	24.77	5.49	3.01	16.44	78
Total post	16.03	16.34	30.78	4.99	3.06	15.03	318
Chi-Squared	P < 0.048						

c. Results of the treatment

The treatment outcomes for the main treatment groups and additional subgroups are displayed in Table 5. The success rate of surgical endodontic procedures was significantly higher than that of non-surgical treatment ($p = .035$). Conversely, the subgroups of teeth that had posts tended to do somewhat better following non-surgical therapy as opposed to surgical treatment ($p = 0.111$). There was a significant difference ($p = .018$) favoring surgical treatment for subgroups without a post.

Results did not appear to be influenced by the gender of the patients ($p = 0.728$) or the kind or group of teeth ($p = 0.708$) [Table 5]. When all

teeth were taken into account, the only variables that exhibited relationships with result in bivariate analysis were the patients' age and the pre-operative PAI score ($p < .001$), aside from the difference between the treatment groups. Table 5 shows that patients under 50 years old had a better prognosis than those over 50 ($p = 0.014$). Table 6, shows that the relationships between age and pre-operative PAI score and outcome were limited to non-surgical cases. However, none of the evaluated criteria demonstrated a significant correlation with the result when the surgical group was inspected independently [Table 7].

Logistic regression analyses of the pooled data identified treatment group, pre-operative PAI

score, age and time to control as significant influencers on outcome. Regression analyses performed on the two treatment groups separately

maintained the association of pre-operative PAI score and age for non-surgical, but not surgical retreatments.

Table 5: Outcomes of treatment for sub-groups of patients (percent)

Category	Success	Uncertain	Failure	No	P-value
All cases	85.06	7.2	7.2	398	
Female	92.4	5.1	2.5	190	
Male	93.3	2.1	4.7	208	0.728
Age < 40	78.21	15.9	5.2	108	
Age 40-50	76.00	16	8.0	103	0.355
Age > 50	68.9	13.55	17.55	187	0.014
Maxillary teeth	74.4	11.6	14	209	0.708
Mandibular teeth	68.9	10.9	20.2	189	
PAI start 3	81.2	1.9	16.9	256	
PAI start 4	67.3	20.0	12.7	82	
PAI start 5	53.1	28.7	18.2	51	<0.001
NSRT	78.3	10.0	11.7	295	
EMS	83.3	7.1	9.6	103	0.035
Post	69.1	16.1	14.7	55	
No post	67.7	17.5	14.9	343	0.781
NSRT post	70.8	17.2	12.0	54	0.111
EMS post	67.6	13.6	18.8	26	
NRST no post	65.3	14.7	16.1	240	
EMS no post	82.5	7.3	10.2	78	0.18
Recall 12 months - <18 months	71.9	12.3	15.8	156	
Recall >20 months	66.9	17.1	16.0	77	0.71

Recall 18 -36 months	67.6	14.6	17.8	102	
Recall > 36 months	66.5	16.2	17.4	63	0.785

Table 6 - Outcomes of treatment for sub-groups of patients – Non-Surgical Retreatment.

Category	Success	Uncertain	Failure	No	P-value
All cases	78.0	9.2	12.8	295	
Female	72.4	11.8	15.8	152	
Male	69.90	14.1	16.0	143	0.821
Age < 40	74.21	15.9	7.02	97	
Age 40-50	72.00	16.5	11.5	105	0.072
Age > 50	66.7	14.53	18.77	93	0.008
Maxillary teeth	69.0	13.0	18.0	163	
Mandibular teeth	66.7	14.6	18.7	32	0.975
PAI start 3	83.2	0.0	16.8	153	
PAI start 4	67.7	17.5	14.8	102	<0.001
PAI start 5	51.1	30.3	18.6	40	
Post	68.2	17.1	14.7	38	
No post	67.6	14.6	17.8	257	0.189
Recall 12 months - <18 months	71.9	12.3	15.8	102	
Recall >20 months	66.9	17.1	16.0	92	0.876
Recall 18 -36 months	66.2	16.0	17.8	65	
Recall > 36 months	66.5	16.2	17.3	36	0.979

Table 7 - Outcomes of treatment for sub-groups of patients –Surgical Retreatment.

Category	Success	Uncertain	Failure	No	P-value
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All cases	83.03	7.1	9.6	103	----
Female	81.4	7.7	10.9	54	
Male	79.8	5.2	15	49	0.455
Age < 40	77.1	14.2	8.7	29	
Age 40-50	75.00	16	9.0	32	0.613
Age > 50	68.9	13.55	17.55	42	0.165
Maxillary teeth	73.3	11.5	15.2	78	0.275
Mandibular teeth	67.5	11.3	21.2	25	0.811
PAI start 3	78.2	10.9	10.9	21	
PAI start 4	66.3	13.7	20.0	07	
PAI start 5	74.5	0.0	25.5	14	0.332
Post	69.1	13.2	17.7	25	
No post	68.2	17.1	14.7	78	0.199
Recall 12 months - <18 months	91.0	3.1	5.9	24	
Recall >20 months	74.4	9.9	15.7	32	0.548
Recall 18 -36 months	71.7	8.0	20.3	34	
Recall > 36 months	71.3	12.6	16.1	13	0.733

Discussion

This study aimed to evaluate the factors and outcomes of treating apical periodontitis that developed or persisted in teeth that had previously had root canal fillings or/and had undergone orthodontic treatment. All cases were examined for non-surgical or surgical retreatment by the principal investigator and later on by the co-investigators. The investigators evaluated the result using the intraoral periapical radiography PAI scoring method. Previous studies have verified the method through comprehensive practice (25, 26). Since there was a high level of

intra-observer agreement on PAI scores, the results can be trusted.

However, when compared to intraoral periapical radiography, cone-beam computed tomography (CBCT) imaging is more sensitive, finding around twice as many periapical lesions (27). Higher radiation levels, however, make it unsuitable for routine clinical case monitoring (28); in addition, clinical studies need to take cost, high levels of scatter and noise, and variations in dose distribution within a volume of interest into account (29, 30). While sensitivity of lesion detection in periapical radiographs is low,

specificity is very high (31), which allows for comparisons across groups. Higher sensitivity in the detection of periapical lesions by CBCT compared with traditional radiographs may not justify the standard use of CBCT imaging in diagnosing periapical lesions (32).

Further, the results of present study showed that the patients under age of 50 years (88.67%) and above 50 years (76.96%) preferred NSRT over EMS (11.3% & 23.04%) whereas, the percentage of EMS was greater in patients >50 years (23.04) compared to <50 years (11.33).

The distribution of tooth types remained unaffected by the gender of the patients, while the elderly (>50 years) age group showed a shift in the proportion of maxillary premolars and molars treated. The tooth types of cases chosen for non-surgical treatment varied significantly from those chosen for surgical treatment: more maxillary anterior teeth (23.30%) and premolars (23.30%) followed by molars (22.33) had surgical treatment, whereas mandibular teeth, particularly molars (32.54%), predominated among teeth treated non-surgically. The percentage of anterior teeth and premolars treated surgically was greater in previous studies (33-35).

Several recent researches have documented the greater percentage of molars being treated surgically compared to the prior studies. The results of present study also showed that maxillary molars (22.33%) underwent EMS which can be a good appraisal to cases maxillary molars (27.79%) treated through NSRT. The rise in the molars being treated by EMS is also subjected to the advancement of methods, materials and operator skills (36, 37).

To compare the outcomes of two separate treatment procedures with two different scoring indexes, the PAI scores at control were grouped

from a full-scale 5-score index to three categories for surgical treatment outcome comparison with Rud's criteria. Categorizing all PAI scores of 3 as "uncertain" could be seen as rigorous because some cases may have improved from an initial score of 4 or 5, indicating healing, and others may have had insufficient observation periods for healing to fully occur. Nonetheless, the criteria for the surgical "uncertain healing" category may also be applicable (23); for this reason, the more rigorous classification was selected for this research evaluation.

The success, uncertainty, and failure criteria applied to surgical cases were derived from the works of Molven et al. (23) and Rud et al. (24). The present study resulted in successful outcomes from surgical retreatment (83.03%), this ratio is comparable to several recent comparable studies (37, 38), but not as good as others (39, 40). According to Molven et al. (23), radiographic assessment of healing ("incomplete" vs. "uncertain") can be difficult. The inclusion of an "uncertain" outcome category suggests the disease development or healing processes may not have completed their full biological cycle. The two modalities may have different underlying mechanisms (41). In this study, four cases out of eight cases were initially "uncertain" (12–36 months) which could eventually heal if longer follow-up would be done. The results of this study are supported by an investigation carried out by Huang et al., where uncertain cases were eventually healed during the 5- to 9-year follow-up period (42). Therefore, longer follow-up times could alter the outcome (43). Furthermore, the lack of calibration criteria for the grading of treatment outcomes following endodontic surgery complicates cross-study comparisons and

highlights the necessity of consistent and standardized follow-up protocols for endodontic surgery patients (39). Longer follow-up periods may also alter the outcome of non-surgical retreatments (40).

Regardless of the radiographic score or treatment group, cases exhibiting radiographic evidence of a sinus tract or vertical root fracture were classified as failures. Both surgically and non-surgically treated teeth may develop vertical root fractures (44). In the present study, only four teeth—two in the surgery group and two in the non-surgical group with posts were removed because of vertical root fractures and were eventually extracted; this data is insufficient to affect the results as a whole.

Challenges for non-surgical retreatments include difficulties with treatment, the quality of the root filling and coronal restoration, and the onset and ongoing existence of asepsis (45). In line with recent findings (46) and systematic reviews of the outcomes following non-surgical retreatment (47, 48), the results of NSRT in present study (78%) obtained were comparable. The literature reports outcomes of surgical retreatment that vary from 67.5% to 92.0% (defined as full radiographic healing (49)). The current study's findings, which showed an 83.03% success rate with EMS, fall in the middle of this range.

Treatment outcome for endodontic surgery has been linked to tooth type, presence and size of a pre-operative radiographic lesion, pre-operative symptoms, coronal restoration, quality of prior non-surgical endodontic treatment, and the presence of an intra-canal post (50). Hemostasis and operator competence are two intraoperative characteristics that are thought to be very crucial (51). In the current study, the influence of operator skill level and related variances in technical quality were somewhat mitigated by supervision and practical instruction from

experienced endodontists. The findings we obtained, that demonstrate no relationship between the listed parameters and the surgical outcome, are consistent with other recent research (52).

Patients were routinely recalled for follow-up consultations following treatment; however, no attempt was made to recall patients in cases where follow-up data was lacking. For cohort studies of this type, the recall rates of 68% and 76% (for the non-surgical and surgery groups, respectively) may be deemed satisfactory (53). Nevertheless, care should be taken when interpreting the data due to the missing data.

The present study also investigated that microsurgery yields superior results than non-surgical retreatment. These findings are in line with findings from another researches (54, 55). A regression of successful endodontic surgery cases over a 4-year follow-up period has been reported in certain studies, although faster recovery following the surgical procedure compared with non-surgical retreatment after 1 year (56). Longer observation periods may cause surgical retreatment to fail again (57), but with the use of contemporary microsurgical techniques and careful case selection, the likelihood of outcome reversals may be reduced.

Teeth with intra-radicular posts typically had superior results when retreated non-surgically (70.8%) than teeth without posts (65.3%). Case selection bias may be a complicating issue; it is most likely caused by the location of the tooth, variations in periodontal health, the quality of prior root canal therapy, and the size and proximity to the intra-radicular post apex. As ultrasonic instrumentation is considered a safe and efficient means of reducing fracture, perforation, and weakening of residual tooth

substance (58), its routine use in the study for post removal is probably responsible for the favorable results of non-surgical retreatment of teeth with posts. The present study also revealed contrasting findings regarding intra-radicular post in cases retreated with EMS, a more favorable outcome was noticed in teeth without post (82.5%) opposed to teeth with post (67.6%). The present study also addressed the ortho-endodontic relationship for retreatment cases with apical periodontitis. Although there is very limited data available on impact of orthodontic treatment on RCT treated teeth, the observations made by radiographs and PAI index resulted in non-surgical or surgical retreatment of teeth which underwent orthodontic treatment due to the fact that the periapical bone destruction is significantly increased and oral health is significantly compromised following orthodontic treatment. However, these findings are supported by previous study Ali Alqerban et.al, which showed that according to the PRI (probability index), the proportion of teeth with periapical bone loss increased significantly after orthodontic treatment. Overall, there was no significant improvement in PAI (peri-apical index) ratings following orthodontic treatment. However, the quality of the endodontic therapy reduced the change in both the PAI score and the PRI which could be due to lack of patient's motivation and adopting adequate oral hygiene practices subjected to prolonged orthodontic treatment (59).

Conclusion

When compared to non-surgical retreatments, this study indicated that surgical retreatments had a better success rate. On the other hand, non-surgical retreatments, as opposed to surgical retreatments, typically yielded superior results

for teeth with intra-radicular posts. While no documented factor influenced endodontic microsurgery treatment outcomes, lower starting PAI scores and younger patient age were associated with successful outcomes for non-surgical retreatments. It is evident that the criteria for selecting cases for surgery and non-surgical retreatments differ, as seen by variations in patient age, tooth groupings, and intra-radicular post presence. Moreover, a longer follow-up periods could alter the results of NSRT or EMS from "uncertain" to "success".

Data Availability: The corresponding author can provide the data that supported the findings of this study upon request.

Conflict of Interests: The authors declare no conflict of interest.

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