



# Post-obturation pain and its relationship with reference time and other risk factors

Dor pós-obturaç o e sua rela o com o tempo de refer ncia e outros fatores de risco

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## ABSTRACT

**Objective:** To evaluate incidence and intensity of postoperative pain in patients treated in the Residency Program in Endodontics at the State University of Maring . The influence of factors inherent to the patient and treatment on preoperative pain was also the focus of our study. **Material and Methods:** 99 patients participated in this study and underwent non-surgical endodontic treatment. Initially, palpation, vertical and horizontal percussion were performed on the tooth to be treated. After the root canals filling, patients received a printed Numerical Rating Scale and were instructed to record the level of pain in the postoperative periods of 24, 48 and 72 hours. For each postoperative period, the patient recorded the values. A descriptive analysis of the data and a model of generalized estimation equations were performed to verify the relationship between the variables and postoperative pain, at a significance level of 5%. **Results:** During the follow-up period, the pain rates found were low represented by 16%, 11% and 7% in the periods of 24, 48, and 72h, respectively. In addition, the rates significantly decreased after 72h. Only one case of *flare-up* was recorded. Presence of pain on palpation, use of reciprocating file and use of 2.5% sodium hypochlorite influenced the results ( $p < 0.05$ ), increasing the changes of post-operative pain by 3.36, 0.4 and 0.2 times, respectively. **Conclusion:** Incidence and intensity of postoperative pain monitored in the residency program reduced significantly after 72 hours. Postoperative pain was associated with pain on palpation, use of reciprocating files and irrigation with 2.5% hypochlorite.

## KEYWORDS

Endodontics; Postoperative pain; Pain measurement; Endodontic treatment; Root canal therapy.

## RESUMO

**Objetivo:** Avaliar a incid ncia e intensidade da dor p s-operat ria em pacientes atendidos no Programa de Resid ncia em Endodontia da Universidade Estadual de Maring . A dor do paciente foi monitorada ap s 24, 48 e 72 horas ap s a obtura o do canal radicular. A influ ncia de fatores inerentes ao paciente e ao tratamento na dor pr -operat ria tamb m foi foco de nosso estudo. **Material e M todos:** 99 pacientes participaram deste estudo e foram submetidos a tratamento endod ntico n o cir rgico. Inicialmente, foram realizadas palpa o, percuss o vertical e horizontal no dente a ser tratado. A presen a de dor foi classificada em uma Escala Num rica de Avalia o (NRS). Ap s a obtura o dos canais radiculares, os pacientes receberam a NRS impressa e foram orientados a registrar o n vel de dor nos p s-operat rios de 24, 48 e 72 horas. A cada p s-operat rio, um pesquisador entrava em contato com o paciente e registrava os valores. Foi realizada uma an lise descritiva dos dados e um modelo de equa o de estimativa generalizada (GEE) para verificar a rela o entre as vari veis e a dor p s-operat ria, ao n vel de signific ncia de 5%. **Resultados:** Durante o per odo de acompanhamento, os  ndices de dor encontrados foram baixos representados por 16%, 11% e 7% nos per odos de 24, 48 e 72h, respectivamente. Al m disso, as taxas diminuíram significativamente ap s 72h. Apenas um caso de reagudecimento foi registrado. Presen a de dor   palpa o, uso de lima reciprocante e uso de hipoclorito de s dio 2,5% influenciaram os resultados ( $p < 0,05$ ),

umentando as alterações da dor pós-operatória em 3,36, 0,4 e 0,2 vezes, respectivamente. O tempo médio de encaminhamento foi de 6,28 meses, o que não influenciou nos resultados. **Conclusão:** A incidência e intensidade da dor pós-operatória monitorada no programa de residência reduziram significativamente após 72 horas. A dor pós-operatória foi associada à dor à palpação, uso de limas reciprocantes e irrigação com hipoclorito a 2,5%.

## PALAVRAS-CHAVE

Endodontia; Dor Pós-Operatória; Mensuração da Dor; Tratamento endodôntico; Escala Numérica de Avaliação.

## INTRODUCTION

The endodontic treatment aims to reverse an installed pathology and eliminate associated signs and symptoms with chemical-mechanical preparation of the root canal and its filling in a hermetic way, allowing the repair of periapical tissues. Studies indicate that after the completion of this treatment painful symptoms may occur in different degrees of severity and could last for a few hours or even continue for days [1], negatively influencing daily aspects such as chewing and stress level, and thus impacting the quality of life [2].

Postoperative pain is defined as a type of pain of any degree that occurs after the start of endodontic treatment [3], and its occurrence varies between 3% and 58% [4]. The continuation of this pain and/or swelling with a severity that disturbs the patient's routine, to the point of needing an emergency consultation, is called flare-up [5], with a frequency of 0.39% to 20% [1,6], and associated mainly with teeth diagnosed with pulp necrosis [7].

This pain originated after endodontic treatment is influenced by numerous factors such as age and gender of the patient [8]; preoperative pain [9]; dental group and dental arch [10]; initial pulp and periapical diagnosis [11]; use of systemic medication before [12] and after [13] treatment; instrumentation [3]; irrigating solutions [14]; intracanal medication [15]; apical patency [16]; working length control [17]; filling cement [18]; filling technique [19]; number of sessions required to complete treatment [20]; and extrusion of debris in the apex region [21].

In the basic units of the Brazilian Unified Health System (SUS), the endodontic treatment offered is of emergency nature, where only an incomplete instrumentation, application of intracanal medication and temporary restoration are performed. The complete endodontic treatment is provided at specialized centers. A referral is necessary for patients to arrive at

specialized centers and the waiting time for care can reach months. Even if a relationship may exist due to the incomplete removal of the infectious content and the loss of the efficacy of the intracanal medication after a long period, no clinical studies were found that compared the referral time with postoperative pain.

Bearing in mind that postoperative pain is a condition that affects a portion of patients after non-surgical endodontic treatment and that its appearance has an impact on their quality of life, the identification of the causal factors is still necessary due to a variation of results in the literature that makes it difficult to conclude about its origin. Knowing what can cause postoperative pain may help the dentist to perform an adequate management during all the operative phases, with the goal of reducing the chances of discomfort to the patients after the endodontic treatment is finished.

Therefore, the main objective of this study was to evaluate incidence of postoperative pain and its intensity after 24, 48 and 72 hours, in patients treated in the Endodontics Residency Program of the State University of Maringá (UEM). The influence of possible factors related to postoperative pain were analyzed as secondary objectives, such as age, sex, fear of dentist, dental arch and group, pulp and periapical diagnosis, the endodontic files used, concentration of the irrigating solution, execution of apical patency, number of sessions, use of intracanal medications, root-filling cement, root-filling technique, temporary sealing material, the operator's level of experience and referral time elapsed between the initial emergency care and the program's care.

## MATERIAL AND METHODS

### Ethics approval

This prospective observational study was approved by the UEM Standing Committee on Ethics in Research with Human Beings (COPEP)

(Approval number 1.693.424). The selected participants who agreed to participate voluntarily in this research signed the Informed Consent Term.

### Eligibility criteria

Included participants were those referred to the Endodontics Residency Program, who underwent non-surgical endodontic treatment, between September 2018 and September 2019, who were able to classify pain using the applied pain scale. Exclusion criteria comprised teeth with root fracture; cases of endodontic retreatment; teeth with open apex; patients who were pregnant or lactating; those who had uncontrolled systemic diseases or medical contraindication for dental procedures; cases of traumatized teeth; patients taking nonsteroidal anti-inflammatory drugs, analgesics and/or antibiotics; and those participants who did not answer the telephone call at any time during the postoperative evaluation (24, 48 and 72 hours).

### Data collection

Data such as age, sex, systemic condition and fear of dental treatment, were collected by the resident through anamnesis, as this information is part of the unique medical record used at the university where the research took place.

The classification of the patient's pain level was measured using a numeric rating scale (NRS), which consists of a ruler, horizontally divided into eleven equal parts, successively numbered from zero to ten. With it, the patient indicated the pain felt, with zero corresponding to "no pain", one to three "mild pain", four to six "moderate pain" and seven to ten "severe pain" (Figure 1).

### Pulpal and periapical diagnosis

After the anamnesis, if fulfilled the inclusion criteria, the physical examination was performed. The palpation of the area was performed with light pressure, both on the buccal and lingual/palatal surfaces. Percussion, on the other hand, was executed with the aid of a clinical mirror

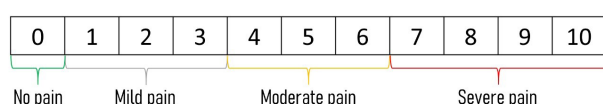


Figure 1 - Numerical rating scale.

handle, where light strokes were made in both vertical and horizontal direction of the tooth. In the cases where the patients presented some painful symptoms by means of the applied tests, the evaluator asked him to give a note in the NRS of pain.

The cold sensitivity test was performed with Endo-Ice (Maquira, Maringá, PR, Brazil) after relative isolation of the tooth with cotton rollers and air-jet drying, applying the product in a cotton ball and then placing it on the vestibular face of the investigated tooth. If any sensitivity was reported by the patient, the test was considered positive.

At last, the resident performed the classification of the patient's pulp and periapical diagnosis. In some situations, cavity testing was necessary to confirm the endodontic diagnosis. Cases where an initial emergency access was performed were classified as previously initiated therapy. Thus, the following categories were adopted (Table I)

### Endodontic treatment

After the initial examination, residents started the endodontic treatment. As it is an educational institution, the treatment protocols performed were varied, however, it was decided to select the cases where manual and/or reciprocating instruments were used, since these are the majority within the program.

The teeth were anesthetized with 2% mepivacaine hydrochloride with 1: 100000 epinephrine using the anesthetic technique recommended for the area of the tooth to be treated, respecting the maximum dose limits. The access to the cavity was executed with the aid of spherical diamond burs and carbide burs coupled to a high-speed dental handpiece. After the exposure of the pulp chamber, the teeth were isolated with a rubber dam and clamp, and cavity's contour and e convenience shape

Table I - Pulpal and periapical diagnosis

Pulpal diagnosis	Periapical diagnosis
Symptomatic irreversible pulpitis	Acute or symptomatic apical periodontitis
Asymptomatic irreversible pulpitis	Acute apical abscess
Pulp necrosis	Chronic or asymptomatic apical periodontitis
Previously Initiated therapy	Chronic apical abscess

were prepared. The length of the canals was determined using an electronic apex locator when the apical patency was performed. When the apical patency was not possible to perform, the length was determined with radiographic aid.

The coronal and middle thirds were prepared with Gates-Glidden drills. Afterwards, the root canals were instrumented using the step-down technique or the step-back technique, according to the resident's choice, using manual and/or reciprocating instruments. During the entire preparation, irrigation of the root canals was performed with the aid of a needle attached to a 5ml syringe filled with sodium hypochlorite in concentrations of 1% or 2.5%.

After the mechanical-chemical preparation of the root canals, 17% EDTA (Maquira, Maringá, PR, Brazil) was applied for five minutes with agitation to remove Smear Layer. Final irrigation was then performed with 5 ml of sodium hypochlorite, and the root canals were dried with absorbent paper tips. In cases where there was presence of exudate, the patient was tired or the consultation time was running out, intracanal medication was used, choosing formocresol when the instrumentation was not finished, calcium hydroxide associated with propylene glycol in cases without apical periodontitis, or calcium hydroxide associated with propylene glycol and camphorated paramonochlorophenol (CPMC) in cases of chronic apical periodontitis.

In sequence, the root canals were filled with gutta percha and the root-filling cement chosen by the resident, which could be Endofill (Dentsply, Pirassununga, SP, Brazil) AH Plus™ (Dentsply, Konstanz, Germany), Sealer 26 (Dentsply, Pirassununga, SP, Brazil), Sealapex™ (SybronEndo - Kerr Endodontics, Detroit, MI, USA) or MTA-Fillapex® (Angelus, Londrina, PR, Brazil), using Tagger's hybrid technique or the single-cone technique. The hermetic sealing was then executed using glass-ionomer cement MaxxionR (Dentsply, Petrópolis, RJ, Brazil) or composite resin.

### Postoperative pain evaluation

After the endodontic treatment was completed, the patients received one printed copy of the pain NRS for each postoperative pain assessment (24, 48 and 72 hours). The participants were instructed again on how to

fill the NRS, and informed that an evaluator would contact via telephone to ask the level of pain at that moment and if medication to relieve pain were needed. A single evaluator made the telephone contact, in which the patients reported the level of pain they were experiencing at the time, and then recorded the results in a corresponding form.

### Statistical analysis

The data were tabulated in Microsoft Excel program (Microsoft Corp., Redmond, WA, USA) and described as a percentage. For the analysis of the association between the variables and the presence of postoperative pain, a Generalized Estimating Equation was used, which is an extension of Generalized Linear Models, selected from the Quasi-Likelihood Criteria under the Independence Model. To select the best model from the variables whose models converged, the *dredge* function was used in the R software v. 3.5.1 (The R Foundation), which made the automatic selection of variables. For this, the variables of greatest interest were fixed, such as referral time, pain on palpation, pain on vertical percussion and pain on horizontal percussion. After choosing the model, variables with a p-value <0.05 were considered significant for the appearance of postoperative pain.

## RESULTS

### Preoperative factors

After applying the eligibility criteria, 99 patients completed the study, 59% female and 41% male, with an average of 41 years. When asked about the fear of going to the dentist, 24.13% of women stated positively while 75.86% said they were not afraid. Among men, 83% said they were not afraid to go to the dentist. Most patients did not presented pain on palpation (78%), vertical (57%) and horizontal (62%) percussion (Table II). As for the pain on palpation around the tooth to be treated, 10% classified it as "mild", 10% "moderate" and only 2% had "severe" pain. For vertical percussion test, 23% classified the pain as mild, 11% as moderate and 8% as severe. For horizontal percussion test, 21% classified the pain as "mild", 11% as "moderate" and 6% as "severe".



## Trans-operative factors

Most of the teeth treated were molars (54.5%), followed by premolars (33.3%), canines and incisors (6.06% each). The most frequent initial pulp diagnosis was previously initiated therapy (93%), followed by pulp necrosis (5%) and asymptomatic irreversible pulpitis (2%) (Table II). Regarding the periapical diagnosis, 37% of the cases had chronic apical periodontitis, while 63% did not have periapical bone rarefaction. To confirm the actual working length, an electronic apex locator was used in 96% of the cases and only 4% were confirmed by periapical radiography (Table III).

During the chemical-mechanical preparation of the root canals, 87% of the teeth were instrumented by the step-down technique, 71% of them with reciprocating files and 29% with manual files. The other 13% were instrumented by the step-back technique with programmed progressive retreat, 92% with manual files and 8% with reciprocating files. The most used irrigating agent was sodium hypochlorite at 2.5% (67%). Apical patency was performed in 96% of the cases (Table III).

Of the total number of patients, 49.5% were treated in a single session. The other 50.5% were treated in more than one session, requiring the use of an intracanal medication. Among them, calcium hydroxide with propylene glycol and CPMC was the most applied (72%), followed by

calcium hydroxide with propylene glycol (18%) and formocresol (10%). which were applied in cases where the chemical-mechanical preparation wasn't completed (Table III).

Of the cements used, Sealer 26 was the most prevalent (38.39%), the single cone technique (49.5%) and Tagger's hybrid technique (48.5%) were the most used for the filling of the root canals. For the hermetic sealing, glass ionomer cement was the material of choice in most cases (85%). The average time for referral of patients between the initial emergency care performed at the health units until treatment at the Endodontics residency program was 6.28 months (Table III).

**Table II** - Preoperative factors

Preoperative factors	Categories	%
Sex	Female	59
	Male	41
Fear of the dentist	Yes	21
	No	79
Pain on palpation	Yes	22
	No	78
Pain on vertical percussion	Yes	43
	No	57
Pain on horizontal percussion	Yes	38
	No	62
Pulp diagnosis	Previously Initiated therapy	93
	Pulp necrosis	5
	Asymptomatic irreversible pulpitis	2
Periapical diagnosis	Chronic apical periodontitis	37
	Without periapical changes	63

**Table III** - Transoperative factors

Transoperative factors	Categories	%
Root canals length measurement	Electronic foramen locator	96
	Periapical x-ray	4
Apical patency	Yes	96
	No	4
Files	Manual file	37
	Reciprocating file	63
Instrumentation technique	Step-back with programmed progressive retreat	13
	Step-down	87
Irrigating solution	1% Sodium hypochlorite	33
	2,5% Sodium hypochlorite	67
Intracanal medications	Formocresol	10
	Calcium hydroxide + propylene glycol	18
	Calcium hydroxide + propylene glycol + CPMC	72
Root-filling cement	Sealer 26	38.39
	AH plus™	30.3
	Sealapex™	17.17
	Endofill	13.13
Root-filling technique	MTA Fillapex®	1.01
	Single-cone	49.5
Hermetic sealing agent	Tagger's hybrid technique	48.5
	Glass ionomer cement	85
Number of sessions	Zinc oxide – eugenol cement	2
	Composite resin	13
Operator	One	1
	Multiple sessions	50.5
Referral time	R1	49
	R2	51
	< 6 months	68
Referral time	> 6 months	14
	Not declared	18

## Postoperative factors

In the first 24 hours, approximately 84% of patients reported no pain, 8% mild pain, 6% moderate pain and 2% severe pain. After 48 hours, 89% had no pain, 6% mild pain, 5% moderate pain and none had severe pain. After 72 hours, the last evaluation, the number of patients with no pain increased to 93%, followed by 3% for mild pain and 4% for moderate pain. Among the 99 patients evaluated, only one reported the presence of edema after filling, with moderate pain, which required an appointment for intervention, being then classified as flare-up. The pain rate decreased significantly between the intervals of 24h to 48h and 24h to 72h ( $p < 0.05$ ) (Table IV). The rate of patients who claimed the need to use pain relief medication was approximately 8%, 5% and 4%, in the periods of 24h, 48h and 72h respectively. Those who did not need medication were 92% in the first period evaluated, 95% in the second period and 96% in the final evaluation.

## Risk factors relation to postoperative pain

Among all the risk factors analyzed, the variables pain on palpation ( $p = 0.021$ ), use of reciprocating file ( $p = 0.026$ ) and concentration of the irrigating agent ( $p = 0.021$ ) were statistically significant ( $p < 0.05$ ), and may be related to the postoperative pain. Thus, pain on palpation before endodontic treatment, instrumentation performed with reciprocating file, and root canal irrigation with 2.5% sodium hypochlorite, increased the chances of postoperative pain by 3.36, 0.4 and 0.2, respectively (Table V). The

other variables were not statistically significant to the appearance of postoperative pain.

## DISCUSSION

In this study, age and sex were unrelated to postoperative pain on this occasion. In contrast with these results, higher pain rates in older patients can be found, which could be justified by the difference in the immune response caused by advancing age [21]. Regarding the sex, there are studies that found higher rates of postoperative pain for the female group and that, when present, the pain manifested itself in greater intensity up to 48 hours after treatment [22]. The higher pain rate found in this group, could be justified by the difference in hormonal rates that they present, mainly modulated by menstrual cycles and use of oral contraceptives [23].

Another element analyzed in the preoperative period was the fear reported by patients regarding dental care, that wasn't a variable associated with the presence of postoperative pain in the present study. The fear expressed in the face of dental care may have been influenced by previous negative experiences, which lead to anxiety in the periods before, during and after treatment, which consequently favors the appearance of moderate to severe pain [24]. In addition to influencing postoperative pain, this anxiety situation causes individuals to avoid regular visits to the dentist, resulting in poor oral health and, when they finally seek dental treatment, the necessary procedures are more invasive, such as endodontic treatment, generating a greater anxiety condition [25].

The presence of pain on palpation in the diagnostic phase was significantly related to the presence of postoperative pain, increasing the chance of development of postoperative pain by 3.36 times. This result was similar to previous studies that display the preoperative pain as a factor associated with postoperative pain [9,26], with intensity ranging from moderate to severe, persisting for up to 48 hours [10,22]. Therefore, patients who are symptomatic prior to treatment should be alerted to an increase in the risk of pain after the endodontic treatment is completed.

Among the 99 teeth selected for this study, 93% of them were previously submitted to urgent treatment and, therefore, were categorized as cases of previously initiated therapy. That means

**Table IV** - Rate and intensity of postoperative pain

Period	Rate of postoperative pain (%)				Total	
	Absence	Mild	Moderate	Severe		
24h	84	8	6	2	16	<i>a</i>
48h	89	6	5	0	11	<i>b</i>
72h	93	3	4	0	7	<i>b</i>

\*Different letters represent a significant difference in the reduction of postoperative pain between the evaluated times.

**Table V** - Factors associated with postoperative pain

Risk factors	OR
Pain on palpation	3.36
Use of reciprocating files	0.4
Use of 2.5% sodium hypochlorite	0.2

the performance of access to the pulp chamber, an incomplete instrumentation (without reaching the entire working length) and application of formocresol as intracanal medication. The use of formocresol as a medication in emergency care was seen as a Brazilian reality, as all cases referred with endodontic therapy previously started were treated with this medication. The lack of a complete instrumentation may be explained by the lack of necessary structure, clinical time available for each patient, or even for the difficulty in instrumenting more complex cases, requiring an endodontic specialist.

In the analysis performed, the referral time was not significantly related to postoperative pain, even though a relationship may exist due to the incomplete removal of the infectious content and the loss of the efficacy of the intracanal medication after a long period. No clinical studies that compared the referral time after previously started therapy until filling with the appearance of postoperative pain were found and, therefore, this would be the first study to address this variable as a possible influence factor on this condition. It is suggested, then, to carry out controlled clinical studies to verify whether this factor has an influence on postoperative pain.

The majority of treated teeth were molars and premolars, mainly using the step-down technique, characterized by its less debris extrusion and contamination deposition to the apical third [26], consequently reducing the chances of postoperative pain [3]. Most cases treated with this technique were instrumented with reciprocating files, which in turn, presented a statistically significant result, increasing the chances of postoperative pain by 0.4 times, when compared to those performed with manual files.

Among the various irrigating solutions, sodium hypochlorite is the solution of choice, due to its antimicrobial capacity and the ability to dissolve organic matter that allow disinfection of the root canals [27]. Sodium hypochlorite in concentrations of 1% and 2.5% was used in this study, with the most concentrated agent increasing the chances of postoperative pain by 0.2 times. The justification for these higher rates of postoperative pain after the use of sodium hypochlorite in higher concentrations would be the toxicity to the periapical tissues that this chemical compound presents [28].

In 72% of the cases that needed more than one session to complete the treatment, it was decided to use calcium hydroxide-based medication associated with propylene glycol and CPMC. This combination is due to biocompatibility [29] and antimicrobial properties of the calcium hydroxide. In the cases included in this study, this association was advantageous, as the microbiota present was unknown.

When assessing the influence of the cements on postoperative pain or the filling technique used, there were no significant results. When there is leakage of cement to the periapical tissues, irritation can be caused, which can influence the postoperative pain [30], but regardless of its composition a remission of symptoms is expected over time [31]. In addition to the irritation caused by the different cements, the filling technique used can contribute to the extravasation of the material and consequently an increase in postoperative pain [32].

Even with a rate of postoperative pain that reached a maximum of 16% among all cases analyzed, reducing significantly after 72 hours, the absence of pain after root canal filling is still sought. Moreover, there is a difficulty in distinguishing the cause of postoperative pain after endodontic treatment, due to the number of factors associated with its appearance, such as patient-dependent factors, trauma during chemical-mechanical preparation, microbiota present, or even caused by the clamp used for isolation, which could mimic pain caused by other factors.

The difficulty in standardizing the participating patients was highlighted as the major limitation to this study, since a previous emergency intervention was performed prior to the complete endodontic treatment in most of the cases treated in the program and, with that, there was a need to diagnosis adequacy, considering cases of previously initiated therapy. Besides, it was not possible to collect the number of emergency interventions that were carried out before definitive specialized care. Thus, the warning of the importance of measures that streamline specialized care, since the average time between initial and specialized emergency care was 6.28 months. Therefore, changes are suggested in the emergency protocols in the Basic Health Units, equipping them with resources (such as x-ray devices and electronic

apex locators) that allow at least a complete instrumentation of the root canals and application of a longer lasting intracanal medications. Or even the hiring of more professionals specialized in endodontics, to be able to meet the great demand of cases with greater agility.

## CONCLUSION

Post-obturation pain can be expected by professionals and patients, even if its onset is in low proportions, significantly reducing its intensity after a period of up to 72 hours. The presence of pain on initial palpation, use of 2.5% sodium hypochlorite and reciprocating instrumentation are possible risk factors for pain after root canal filling.

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## Author's Contributions

ACGA: Responsible for the study design and data collection. LC: Formal analysis, methodology, visualization, writing – review & editing. NNOP, MSE: Advisor. IVM, BASV: Participated in the care of patients in the residency program in endodontics during the research. PS, LFP: Responsible for the statistics.

## Conflict of Interest

There was no conflict of interest.

## Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) and State University of Maringá - Brazil.

## Regulatory Statement

This prospective observational study was approved by the UEM Standing Committee on Ethics in Research with Human (COPEP). The approval code for this study is: 1.693.424.

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