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Periapical lesions after endodontic treatment

Nicolas DIWUY

Dissertação conducente ao Grau de Mestre em Medicina Dentária (Ciclo Integrado)

Gandra, 17 de setembro de 2022

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Trabalho realizado sob a Orientação de Prof. Doutor Paulo Miller

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Eu, **Nicolas DIWUY**, declaro ter atuado com absoluta integridade na elaboração deste trabalho, confirmo que em todo o trabalho conducente à sua elaboração não recorri a qualquer forma de falsificação de resultados ou à prática de plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria do trabalho intelectual pertencente a outrem, na sua totalidade ou em partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores foram referenciadas ou redigidas com novas palavras, tendo neste caso colocado a citação da fonte bibliográfica.

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Eu, Paulo Manuel Cruz Miller, com a categoria profissional de Professor Auxiliar do Instituto Universitário de Ciências da Saúde, tendo assumido o papel de Orientador do Relatório Final de Estágio intitulado "Periapical lesions after endodontic treatment", do Aluno do Mestrado Integrado em Medicina Dentária, Nicola Diwuy", declaro que sou de parecer favorável para que o Relatório Final de Estágio possa ser presente ao Júri para Admissão a provas conducentes à obtenção do Grau de Mestre.

Gandra, 27 de setembro de 2022

O Orientador

ACKNOWLEDGMENTS

To my family, for their support. They have always believed in me and encouraged me even when I wasn't following the proper path.

To my friends, those people who have brightened and enriched these years of study with emotions and smiles. I know you will always be at my side. Words will never be enough to express my gratitude and my feelings.

To my friend and roommate Moustansir, a good and wise man. The moments we lived together will remain wonderful memories, thank you.

To my work partner and friend Adam, for his smile and his humanity.

To my supervisors, Professor Paulo Miller and Professor Antonio Ferraz, for their help and kindness.

ABSTRACT

Background: Inflammation of a tooth can lead to periapical disease. The main objective of endodontic treatment is to clean and fill the root canal to heal the periapical inflammation. The treatment is not 100% successful, and knowledge of the lesions must be thorough.

Aim: The aim of this study is to observe the dynamism of periapical lesions undergoing endodontic treatment.

Materials and methods: For the realization of this integrative systematic review, we did a bibliographic search on the platform PUBMED. After applying the inclusion and exclusion criteria, we have obtained a corpus of 11 studies.

Results: 5 studies (45,5%) were about composition of periapical lesions, 3 studies (27,3%) were about progression of periapical lesions, 2 studies (18,2%) were about the measures of periapical lesions, 1 study (9,1%) was about the relationship between treatment and periapical lesions.

Discussion: Bacteria and microorganisms in the infected area have a strong association with the dynamism of a lesion. Studies show that bacteria are associated with several symptoms and pathogenicity. The probabilities made on the progression of the lesion is important to judge the endodontic treatment and to understand the possible causes of failures. Different techniques are used to quantify the microorganisms in the lesions and study their measurements.

Conclusion: Improved techniques for the study and radiology of lesions will allow for more effective treatment and early diagnosis, thus avoiding pain and other strains on the patient and the practitioner.

Keywords: *Endodontic treatment, lesion post endodontic treatment*

GENERAL TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABREVIATIONS

PAP - primary apical periodontitis

PTAP - post-treatment apical periodontitis

LPS - lipopolysaccharides

LTA - lipoteichoic acid

CBCT - cone beam computed tomography

PAI - periapical index

DNA – Deoxyribonucleic Acid

Td - Treponema denticola

Ef - Enterococcus faecalis

AP - apical periodontitis

PCR - Polymerase Chain Reaction

RNA – Ribonucleic Acid

RT-PCR - Real-time polymerase chain reaction amplification

Ct - cycle threshold

CT - computed tomography

1 INTRODUCTION

Teeth have two basic elements: a crown and one or more roots. The crown of a tooth is typically that which is clinically evident in a patient's mouth in the absence of periodontal bone loss. To be precise, the exact boundary between the crown and the root(s) of a tooth is the cementoenamel junction. The physical composition of teeth is made up of 4 dental tissues: enamel, dentin, cementum, and pulp. The first 3 dental tissues are mineralized hard tissues, while the pulp is a non-mineralized soft tissue. [1]

The dental pulp, which is surrounded by dentin, sustains the vitality of the tooth by providing vital factors through the apical foramen and is a key factor in the maintenance of the tooth. Through the apical foramen, blood vessels supply nutrients and remove waste products, and the neural network indicates the presence of pain stimuli. [2]

The inflammatory by-products of pulpal origin may permeate through the apex or through smaller canals in the apical third of the root canal system and exposed dentinal tubules which in turn trigger an inflammatory vascular response in the periodontium. [3]

In the initial phase of infection, dentin and enamel are the primary barrier to infection. The inflammatory reaction caused by the micro-organisms restricts blood flow to the pulp and reduces its ability to react to the microbial attack, resulting in irreversible pulp damage and pulp necrosis. Root canal treatment is the treatment of first choice for necrotic pulp and apical periodontitis. [4]

The main objective of endodontic treatment is to clean and shape the root canal system restoring and maintaining the health of the periradicular tissue. This is allowed by eliminating the infection from the inner root canal system and prevent re-infection by obturation.[5,6] Microbial invasion and subsequent infection of root canal systems is crucial for the initiation and progression of periapical lesions. The persistence of micro-organisms in the root canal system can lead to an inflammatory and immune response in the periapical tissues, resulting in local bone destruction.[7] This may result in endodontic retreatment or apical surgery to treat persistent lesions or those that appear after the first treatment.

Periapical lesions are one of the most frequent diseases affecting periradicular tissues.[8] They can be very dangerous in patients who do not complain or tend not to visit the dentist. The pathogenesis of apical periodontitis is quite complicated and includes a highly diversified microbiota, virulence factors and immune response. [9] The types of bacteria found range from anaerobic to aerobic, to the most resistant species able to survive in environments without nutrients.[5] The environmental dynamics of these various ecological niches dictate the predominant microorganisms and their potential to affect disease persistence.[3] The virulence of bacteria is due to their virulence coefficients, which are based on the structures, products or strategies used to infect the host. [10]

2 OBJECTIVES

The aim of this study, by means of a systematic review, is to observe the progress of periapical lesions after endodontic treatment, to define them and compare with periapical lesions untreated.

3 MATERIALS AND METHODS

For the realization of this integrative systematic review, we did a bibliographic search on the platform PUBMED using the following combinations of keywords: (***Endodontic treatment AND lesion post endodontic treatment)***

The criteria of inclusion were: 10-year time range, studies only made on humans and studies in English.

The criteria of exclusion were: studies which area of interest was not the periapical lesions, studies which using other treatments, studies not in vivo, studies involving specific pathologies or affections.

The search with the afore mentioned keywords resulted in 308 articles. After applying the inclusion criteria, 191 articles remained. After applying the exclusion criteria, upon reading title, abstract and the article, we decided to select 11 articles. (The flow chart of study

selection for our review is indicated figure 1)

For the introduction and the discussion, we used 12 additional references, found on pubmed, we judged interesting to complement our study.

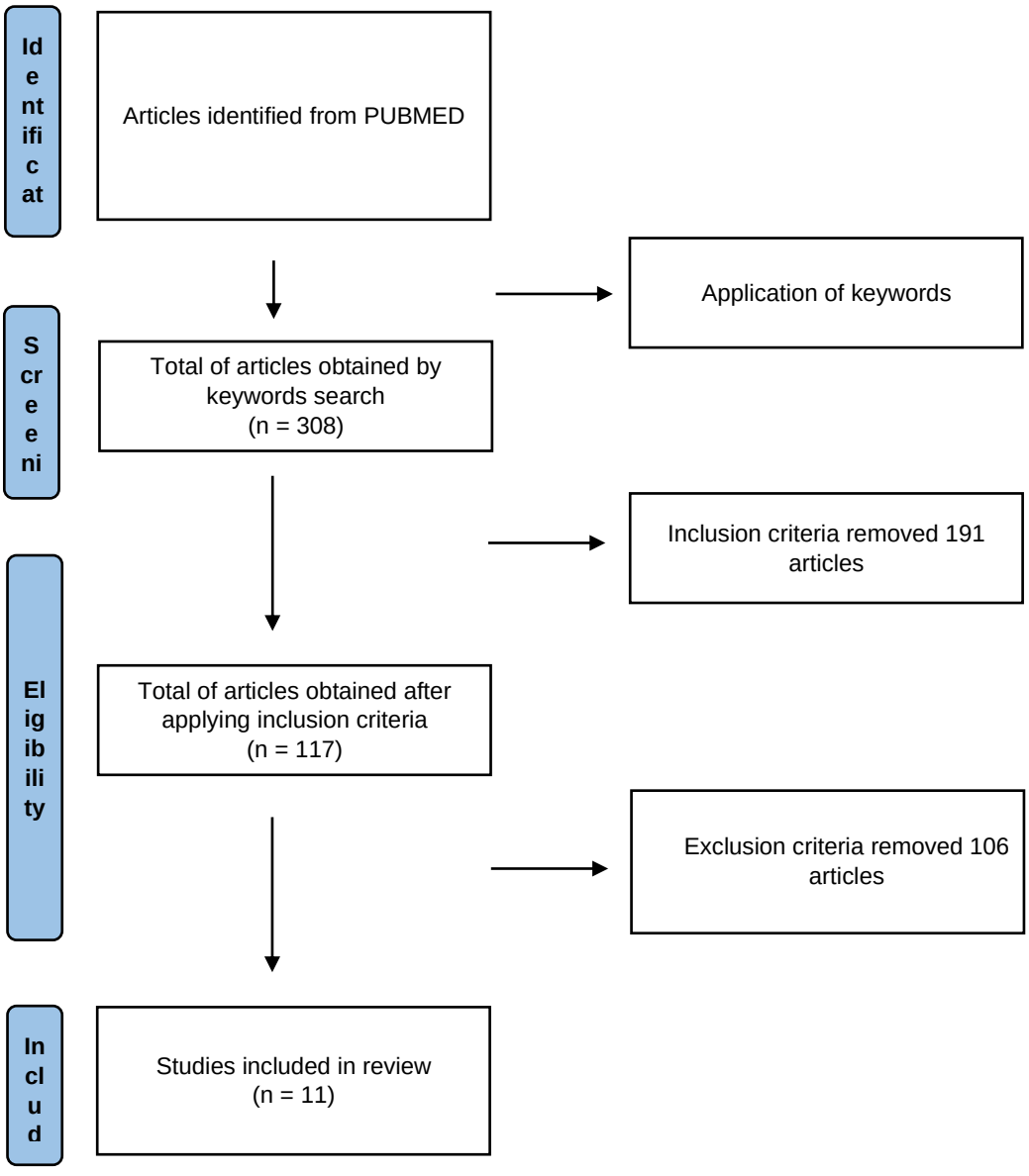

Figure 1: Flow chart of study selection for the review

4 RESULTS

5 studies (45,5%) were about composition of periapical lesions: 2 were comparing micro-organisms and bacteria flora before and after endodontic treatment and 3 were analysing composition of root canal treated teeth with apical periodontitis.

3 studies (27,3%) were about progression of periapical lesions using dental records, radiographic and clinicals data on root canal treated teeth during a defined period.

2 studies (18,2%) were about the measures of periapical lesions: 1 was more general and analysed several dimensions after endodontic treatment and the mucosal thickening and 1 was centred on the volumetric changes after endodontic treatment using CBCT images.

1 study (9,1%) was about the relationship between treatment and periapical lesions using descriptive analysis, and the chi-square test on digital radiological files.

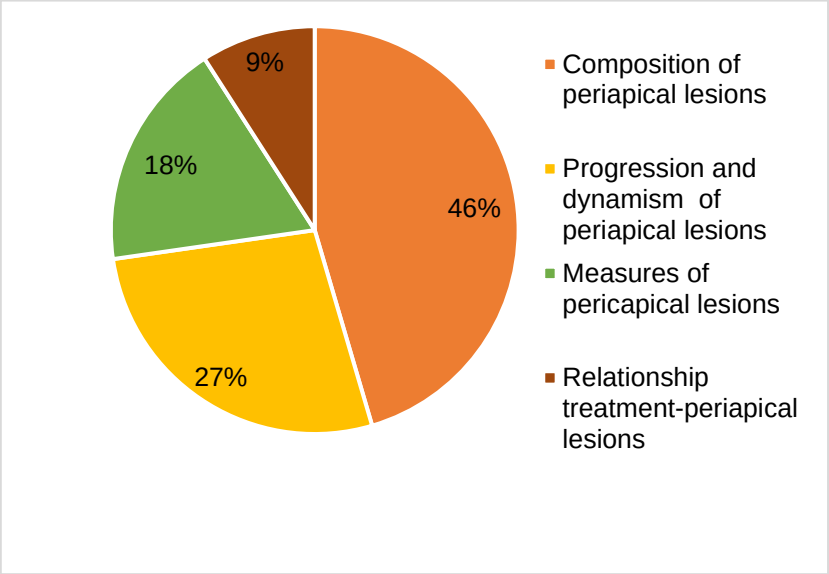

Figure 2: Distribution of the corpus according to the lesions purpose

Study	Purpose	Methods	Population	Results	Conclusion
Felipe Paiva Machado and al. (2019) [9]	Comparison of apical periodontitis composition	Compare the microbial load and composition of primary apical periodontitis (PAP) and post-treatment apical periodontitis (PTAP) and observe the concentration of lipopolysaccharides (LPS) and lipoteichoic acid (LTA). And correlate with clinical signs, symptoms and periapical lesion volume (PLV)	Sixty patients referred for endodontic treatment with PAP (31) and PTAP (29)	A higher number of cultivable bacteria and LPS were found in PAP ($p<0.05$). The median number of species per root canal found in PAP was 9 and PTAP 22. LTA levels were similar in both infections and had no correlation with signs and symptoms.	This study enables the identification and comparison of the different bacteria present in PAP and PTAP. Both infections had a polymicrobial nature with domination of gram-negative bacteria, and PTAP had a greater variety of bacteria.
Victoria Soo Hoon Yu and al. (2012) [11]	Comparison of lesion progression	Data were obtained from interview, dental files, clinical exams, and radiographic examinations	Patients (1214) of a university clinic with persistent endodontic lesions (6 months - 4 years) since treatment and with original treatment radiographs available	Most of the lesions (107, 70.9%) received treatment 4-5 years ago. Eighty-six lesions (57.0%) improved, 18 (11.9%) were unchanged, and 47 (31.1%) deteriorated since treatment.	A specific time interval alone should not be used to conclude that a lesion will not resolve without intervention. This study identified several clinical factors that are associated with deteriorating persistent lesions, which should aid in identifying lesions that require further intervention

Kamburoglu K and al. (2017) [12]	Comparison of lesion dimensions	Width, height, surface area, and volume measurements of periapical lesions and mucosal thickening of the maxillary sinus mucosa in the vicinity of the periapical lesion were measured before and 1 year after endodontic treatment	21 patients (14 female and 7 male) with periapical lesion that had local mucosal thickening in the vicinity of the periapical lesion were endodontically treated.	We found statistically significant differences between mean pre-treatment and mean post-treatment measurements conducted by using CBCT images	We found significant reduction in periapical lesion width, lesion height, surface area, and volume in maxillary molar teeth along with adjacent sinus mucosal thickening by using CBCT and specific software 1 year after endodontic treatment.
Ming-Ming Zhang and al. (2015) [13]	Comparison of volumetric changes one year after endodontic treatment	Evaluation of teeth 2 years after treatment. Two examiners independently measured the volume of radiolucency on CBCT images on two occasions. A Wilcoxon signed rank test was used to assess the volumes 1 and 2 years after treatment.	93 single-rooted teeth 1 year after endodontic treatment	The overall size of the radiolucency significantly decreased during the second year (P = .01); the volume decreased in 38 teeth (63%), remained unchanged in 20 (33%), and increased in 2 (3%).	The volume of post-treatment periapical radiolucency detected 1 year after treatment was significantly reduced after the second year in 63% of teeth.

J. D. Bronzato and al. (2020) [10]	Comparison of apical periodontitis composition after endodontic treatment and retreatment	Clinical and radiographic data were collected. LPS and LTA levels were determined using horseshoe crab amoebocyte lysates and enzyme-linked immunosorbent assays, respectively. Student's t-test or Wilcoxon-Mann-Whitney tests were applied to compare LPS and LTA data with clinical and radiographic features.	Patients with periapical lesions in teeth with post-treatment endodontic disease following primary root canal treatment (n = 19) and unsuccessful root canal retreatment (n = 13)	Parvimonas micra was the most commonly detected species in all groups, followed by Enterococcus faecalis, Fusobacterium nucleatum and Porphyromonas endodontalis.	Periapical lesions associated with teeth after primary root canal treatment and retreatment had similar polymicrobial composition.
Igor Tsesis and al. (2013) [14]	Investigate the influence of various factors on the dynamics of lesions	The dynamics of the included periapical lesions was evaluated based on the periapical index (PAI) score changes between 2 consecutive periapical surveys of at least a 4-year interval.	74 patients with a total of 200 endodontically treated teeth having periapical lesions that fulfilled the inclusion criteria.	Fifty-seven (28.5%) lesions remained unchanged, 103 (51.5%) lesions worsened (PAI score increased), and 40 (20%) lesions improved (PAI score decreased). Poor root canal filling and poor restoration were found to adversely affect the long-term dynamics of the periapical lesions ($P < .05$).	Poor root canal filling and poor restoration may adversely affect the long-term dynamics of periapical lesions that are left without intervention in endodontically treated teeth.

Juan Wang and al. (2012) [15]	Investigate the primary bacterial flora and the localization of extra radicular biofilm in persistent apical periodontitis lesions	Five samples were examined for the presence of biofilm by scanning electron microscopy. Another 5 samples were examined for the presence of biofilm by Brown and Brenn-modified Gram staining. The DNA from 13 samples was processed for amplification via polymerase chain reaction and separated with denaturing gradient gel electrophoresis. Selected bands were excised from the gel and sequenced for identification.	31 patients (12 men and 11 women; mean age, 42.0 years) were enrolled in this study	The following species were detected in the microbial community from the apical samples: Actinomyces sp. oral, Propionibacterium, Prevotella sp. oral, Streptococcus, Porphyromonas endodontalis, and Burkholderia. The prevalence of Actinomyces sp. oral and Propionibacterium were highest (84.6% and 61.5%, respectively).	A multibacterial community, including Actinomycesand Propionibacterium, seems to participate in the maintenance of persistent periapical pathology.
Victor DJ and al. (2021) [3]	Evaluation of persisting pockets with endodontic origin	Subgingival plaque samples were collected from fifty patients diagnosed with a primary endodontic and a secondary periodontal lesion that persisted even after completion of the root canal treatment.	50 patients were recruited into a single group of 18–60 years	The mean cycle threshold value for Treponema denticola (Td) was found to be 33.74, and for Enterococcus faecalis (Ef), it was 34.39. With regard to clinical attachment loss, Td ($P < 0.04$) and Parvimonas micra ($P < 0.05$) had a significant correlation.	Ef (92%) and Td (86%) were found to be most prevalent. Porphyromonas gingivalis and Tannerella forsythia were in minimal to non-existent levels.

Sisko Huumonen & Dag Ørstavik (2013) [16]	Comparison of periapical status after endodontic treatment with preoperative radiographic status	The periapical status was evaluated blindly using the PAI scoring system. The longest follow-up period was 4 years; intervals between controls varied from 3 months to 1 year	1,410 teeth in seven prospective clinical studies	Teeth with preoperative PAI score 1 maintained excellent periapical health throughout. Teeth with preoperative PAI score 2 showed some impairment in health over the first 6 months but improved to approach 95 % healthy teeth at 2 years of observation. Teeth with PAI 3–5 at the start showed significant improvement at 3 months; 27 % were considered healthy (PAI 1 or 2) increasing to 41 % after 1 year. Improvement of periapical status was slower in PAI groups 4 and 5 compared with PAI 3 during the first year.	Healing of pre-existing periapical lesions is most pronounced from 3 months to 2 years

Fabio M Pedro and al. (2016) [17]	Investigate the association between the quality and prevalence of root canal treatment and the apical periodontitis	Data were statistically analysed using descriptive analysis, and the chi-square test was performed with 5% significance level.	1977 digital radiological files (age of the study population was 34.9 years)	Endodontic treatment that showed no periapical changes was 47.7% The periapical lesions were observed in 45% of cases and related to inadequate filling quality. The apical limit was considered inadequate and related to periapical changes in 42% of cases. Periapical changes were present in 52% of cases, regardless of the quality of the filling and apical limit.	It can be concluded that apical periodontitis (AP) is associated with the quality of endodontic treatment.

Pereira R.S. and al. (2017) [6]	Investigate the microorganisms at the root ends and in the surrounding peri radicular lesions	All patients had at least one tooth with a performed satisfactory endodontic treatment, between 1 and 15 years prior to enrolment. The characteristic radiographic evidence of peri radicular bone destruction of post-treatment apical periodontitis was observed in all selected asymptomatic first molar (anterior, posterior, inferior or superior).	Thirty patients (17 female and 13 males) (16 – 58 years)	In both the root end and periapical samples, Fusobacterium nucleatum (71,6%), Dialister pneumosintes (58,3%) and Tannerella forsythia (48,3%) were the most prevalent species. Dialister pneumosintes showed a statistically significant concentration followed by F. nucleatum and T. forsythia, which also showed significantly higher values regarding the other bacteria. Extra-radicular and/or intra-radicular infections were present in all teeth with failed endodontic treatment, and showed polymicrobial infection in most cases, with a predominance of F. nucleatum, D. pneumosintes and T. forsythia.	The bacterial array associated with the 3 mm root ends and peri radicular lesions in post-treatment apical periodontitis are complex and with a high inter-individual variability.

Table 1: Relevant data gathered from included studies

5 DISCUSSION

5.1 Endodontic treatment

It has been considered that more than 50% of tooth mortality is due to endodontic and periodontal problems therefore periodontal and endodontic pathways are critical in deciding which cases will be refractory to conventional endodontic or periodontal treatment. [3,18] The main objective of endodontic treatment is to clean and shape the root canal system restoring and maintaining the health of the periradicular tissue.[5]

Endodontic treatment is a reasonably predictable procedure with success rates of between 86% and 98%.[19] The quality of root canal treatment is influenced by the knowledge, attitude and competence of the practitioner; awareness of the epidemiological factors and consideration of diagnostic and prognostic factors are essential for preventive measures, it may affect outcome and effectiveness of the treatment.[17,20] Radiological identification of periapical disease is important to provide the endodontist with immediate and appropriate dental treatment, the choice to operate on a tooth with periapical radiolucency must be based on the technical feasibility of the treatment, factors such as patient age, tooth type, smoking, periodontal history, severity of lesions, the patient's values and preferences.[12,14,20]

The identification of microbial virulence factors has allowed clarification of the infection's pathogenesis, it underlines the importance of root canal treatment for the healing of apical periodontitis (AP), because a periapical lesion will not heal on its own, this healing also depends on the host immune system's response to the biofilm to eliminate staying in the root canal system or surrounding the apex, two places with dynamic processes that can change over time, the host immune is the first line of microbial elimination in the periapical region but this immune response is not sufficient, as microorganisms with a very high capacity of adaptation and survival can, on a long-standing infection, grow and block the periapical cure. [6,10,13] Indeed, the apical third of the root canal has a low oxygen tension and a rich presence of proteins and glycoproteins, which are inducers for the growth of oral anaerobic bacteria.[6]

The endodontic treatment first involves access to the tooth's canals through a central opening of the pulp chamber, followed by pulp removal, cleaning, disinfection, drying and shaping of the canals with files. This step is done manually or, more often now, with a

powered technique. Once the canals have been prepared, sealing is done by blocking with gutta cones and root canal cement. These last two elements mould the inner anatomy of the tooth. It is imperative to fill to the end of the root to ensure watertightness, which requires verification of the quality of the filling via an apical radiography. The endodontic treatment can be done in multiple session, temporary sealing during treatment and definitive restoration is crucial, during this time all care supplied in the clinical process can be compromised. Also, the final restoration after endodontic treatment is a critical factor in the effectiveness of the therapy, bacterial components can pass in the canal through the filling from the coronal to the apex after a time of exposure to saliva, biofilm, and microorganism.[17]

In the study of *Fabio M Pedro and al.* it can be concluded that the premolars show the highest incidence of endodontic treatment, followed by the molars, canines are the teeth requiring the less amount of endodontic therapy. Fabio M Pedro and al. also compared the maxillary and the mandibular, they indicate that inferior incisors and canines have the lowest rate of endodontic treatment, and the superior premolars and incisors have the highest incidence.

The higher frequency of treatment on the posterior teeth may be due to the reason that these teeth, being at the back of the mouth, do not participate in the smile, do not constitute the esthetic part, and are not properly cared. The absence of appropriate dental health suggests that posterior teeth are more susceptible to the impact of micro-organisms in the oral cavity, resulting in coronal damage (caries) and endodontic problems, we can also notice that maxillary and mandibular canines and maxillary second premolars have the better prognosis for recovery after a treatment. [16,17]

5.2 Treatment failure

Endodontic treatment is not a therapy without failure, its success depends upon: removal of bacteria from infected root canals and control of secondary infection. However, failure should not be considered if the size of the lesion decreases, or as long as the lesion is asymptomatic, this raises an important issue, the judgement of whether a lesion is healing or deteriorating, all the clinical, radiographic data are needed to establish the status of the lesion to decide if retreatment is required or not.[11,16]

The complex root canal anatomy composed of many dentinal tubules, which are microscopic tubular spaces contained within the dentin and extending from the pulp-dental margin to the enamel margin, makes the complete elimination of microorganisms and biofilm residing in the root canal system impossible, in addition, microbial biofilms are inherently resistant to antimicrobial agents adapt by activating survival genes and using alternative metabolic pathways, have the capacity for bacterial aggregation and synergy, and are located in areas where nutrient sources are little affected by endodontic treatment.[13,15,19]

Premolars and molars have a higher failure rate; this must be because premolars and molars present more anatomical variations and root ramification patterns. The morphology of the root canal of these teeth is complex and variable, which makes endodontic treatment more difficult and therefore reduces the predictability of the prognosis, these variations represent a source of infection and a major cause of treatment failure, which can lead to complications such as the decision to extract in case of retreatment that is difficult to perform. Most failures happen when treatment procedures, mainly of a technical reason, do not reach adequate levels of infection clearance and reduction. [17]

Igor Tsesis et al (2013) emphasised the importance of the quality of root canal filling and coronal restorations in relation to AP, as poor filling has a negative effect on the long-term dynamics of periapical lesions, lesions left untreated deteriorate and enlarge, causing new symptoms and suffering in the patient. *Fabio M Pedro et al* (2016) also reveal a correlation between the existence of periapical changes and the quality of the filling, in 45% of the cases of a poorly performed filling negative periapical changes occurred, while in the opposite case of an adequate filling only 7% of the lesions had negative dynamics. These data show us that endodontic treatment is indispensable for the care of AP.[14,17] While the root filling is the primary safeguard against periapical infection, over time the influence of additional factors, such as inadequate or deficient coronal restoration, can lead to root canal infection and alter the treatment outcome and lesion dynamics.[16]

The apical anatomy includes a constriction, located on average 0.5 to 1.5 mm from the root apex where the foramen is located, it is considered the narrowest part of the canal and is the most commonly reference junction used as an apical termination for shaping, cleaning and filling. [17,21] One of the main failures of endodontic treatment is the opening of the apices which can be due to apical root resorption caused by periodontitis or chronic apical trauma

and excessive instrumentation, because the apical areas are irritated and mechanically breached, which widens the apical constriction, leading to opening of the apices.[21]

5.3 Lesion

5.3.1 Bacteria and microorganisms

To improve the success rate of endodontic treatment we need to study the lesions, their microorganisms and the instrumentation techniques. For the study of the bacterial flora, in the areas where the biofilm and microorganisms that form and deteriorate periapical lesions reside, several techniques exist:

- Polymerase Chain Reaction (PCR), which is an extremely sensitive molecular technique used to identify endodontic pathogens that are difficult to culture, without the need to culture them. It allows to obtain, from a complex and scarce sample, large quantities of a specific DNA fragment of defined length. The principle is to carry out a succession of replication &reactions of a double-stranded DNA template. Each reaction uses two oligonucleotide primers whose 3-prime ends point towards each other. The "primers" define the sequence sought.[6,10]
- Culture techniques which consist of taking samples from the root canals using paper points, the samples are then agitated in a vortex for 60s and then distilled in series (1/10, 1/100 and 1/1000). The dilutions are then plated onto agar using sterile plastic spacers and incubated at 37°C in an anaerobic atmosphere for 14 days allowing quantification of colonies in CFU/mL.[9]
- Checkerboard DNA-DNA hybridization is a technique that provides a simultaneous quantitative analysis of 40 microbial species against up to 28 mixed microbiota samples on a single membrane; using digoxigenin (DIG)-labeled, whole-genome DNA probes. This technique is used with the culture procedure.[9]
- A technique using the SYBR Green system, a fluorophore, allows radioactive labelling for a wide range of methods, including the detection of DNA and RNA on polyacrylamide or agarose gels. It can also be used to quantify nucleic acids in

solution and, as it interferes little with the polymerase chain reaction, it is the main sequence marker used in real-time PCR.[6,15]

- Real-time polymerase chain reaction amplification (RT-PCR), a reaction is detected by the appearance of a fluorescent signal. The cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to be detectable. Ct levels are inversely proportional to nucleic acids, it's mean lower the Ct level is, higher the amount of nucleic acid is[3]. The scale of detection applied in this study is:
  - Cts ≤29 abundance, strong reaction
  - Cts of 30-37 moderate amounts
  - Cts of 38-40 minimal amounts[3]

The study of *Felipe Paiva Machado and al.* compared the microbiota found in PAP (primary apical periodontitis) and PTAP (post-treatment apical periodontitis), observing levels of lipoteichoic acid (LTA) and lipopolysaccharides (LPS), using samples collected with paper points in culture identified with checherboard DNA-DNA hybridization. When the bacterial flora of the different infections (PAP and PTAP) was compared, the data showed a more significant number of CFU/mL in PAP compared to PTAP. The most prevalent microorganisms in PAP were *P. gingivalis* and *S. intermedius* (45.16%), *E. faecalis*, *P. acnes*, *C. sputigena* and *P. melaninogenica* (41.94%), *L. buccalis* (38.71%), *S. constellatus* (35.48%) and in PTAP were *P. gingivalis* (72.41%), *C. rectus* (68.97%), F. nucleatum and E. nodatum (65.62%), *S. mitis* (62.07%), *E. faecalis*, *S. constellatus*, *E. faecium*, and *E. saburreum* (58.62%). E. faecalis was one of the most common species present in apical periodontitis, in 41.94 and 58.62% of the samples collected in the PAP and PTAP, respectively. *Porphyromonas gingivalis* had the highest frequency in both infections, this pathogen interacts with *F.nucleatum*, an invasive, adherent and inflammatory bacterium, which contributes to its colonisation. They concluded that there was a large interaction between the species, the levels of LTA were not the same before and after treatment and were not related to symptoms or periapical lesion volume. LPS levels were higher in PAP and are related to periapical lesion volume.[9]

In the study of *J. Wang and al.,* the microbial community in the apical samples included *Actinomyces* sp. oral, *Propionibacterium*, *Prevotella sp*. oral, *Streptococcus*, *Porphyromonas*

endodontalis and *Burkholderia*, with the most prevalence of Actinomyces sp. oral and Propionibacterium (84.6% and 61.5%, respectively). Biofilms were observed on the root surface, extracellular material held the bacteria together, and the biofilm was composed mainly of cocci and rods.[15] *Actinomyces* and *P. propionicum* are present in persistent and secondary infectious lesions, their presence has also been demonstrated in asymptomatic lesions resistant to endodontic treatment.[15]

In the study of *J. D. Bronzato and al*., in periapical lesions, *Pavimonas micra* called *P.micra* was the most prevalent species with 87.5% prevalence, E. faecalis was detected with 75% prevalence and *F. nucleatum* was the third most abundant bacteria with 68% prevalence. They also compared the level of endotoxins (LPS) and lipoteichoic acid (LTA) present in periapical lesions and obtain levels of LPS significantly lower than those of LTA in the periapical lesions of treated and reprocessed teeth, indicating the excess of Gram-positive bacteria over Gram-negative bacteria in secondary or resistant periapical lesions.[10] They also concluded that TLR4s in nerve cells can link to lipopolysaccharidique (LPS) wich is endotoxins, and subsequently liberate neuropeptides that are associated with pain. LPS can cause an inflammatory response by releasing pro-inflammatory cytokines.[10]

The study of *Victor DJ and al.* has shown that *Enterococcus faecalis* was present in 92% of the samples, *Treponema denticola* in 86%, *Prevotella intermedia* and *Porphyromonas gingivalis* in 56%, *Parvimonas micra* in 34% and *Tannerella forsythia* in 26%. They used a RT-PCR technique, the most important Ct values were Td with 33.74 ±2.99, and Ef with 34.39 ±2.10. Other bacteria that were evaluated had a mean level of expression with Ct values >37, indicating a level of the bacterial nucleic acid ins ignificant.[3]

In the study of *Pereira R.S. and al.,* the bacterial population in root canal and periradicular tissues were, in decreasing order of prevalence, of *F. nucleatum* (71.6%) > *D. pneumosintes* (58.3%) > *T. forsythia* (48.3%) > *A. actinomycetemcomitans* (25%) > *T. denticola* (16.6%) > *P. intermedia* (15%) > *P. gingivalis* (15%) > *E. faecalis* (11.6%) > *P. endodontalis* (10%) > *Prevotella nigrescens* (1.6%). The technique for bacteria quantification was Real-time PCR using SYBR Green system and the samples were collecting during apical microsurgery.[6]

P.gingivalis is an anaerobic Gram-negative bacterium, which exhibits virulence factors such as the induction of pro-inflammatory cytokines, the production of enzymes and proteins that allow it to evade host defences and the presence of fimbriae. Fimbriae are outgrowths of the outer membrane that allow it to move and adhere to an organic substrate. *P.gingivalis* is one of the main factors in the biofilm that leads to pathological bone loss. It is also associated with systemic diseases such as diabetes, cardiovascular diseases and orodigestive cancers. [22]

The ability of *Actinomyces* and *Propionibacterium* to survive and adhere to the extraradicular zone contributes to the development of infection. Both types of bacteria have fimbrial structures and therefore possess the functions associated with fimbriae:

- adhesion to dentinal debris expelled through the apical foramen as it progresses through the periapical tissues,
- fixation to other bacteria or host cells,
- attachment to the surface of the root apex,
- coaggregation to other bacteria [15]

Actinomyces are chemotactic (movement of an organism or entity in response to a chemical stimulus), stimulating lymphocyte blastogenesis and releasing lysosomal enzymes from polymorphonuclear leukocytes and macrophages. Propionic bacteria are gram-positive, clustered and can grow between 15°C and 40°C, their original habitats can be skin and dairy products. [15]

P. micra is a gram-positive anaerobic coccus, they possess several virulence factors:
its cell wall triggers the secretion of TNF-α, IL-1β, IL-6 and IL-8, which induces bone resorption and AP formation. [10] In contact with endothelial cells, activates plasmin and increases tissue penetration associated with the disruption of the composition and abundance of common microorganisms established in the biofilms that cover subgingival plaque.
P. micra can cause infection at other sites in the human body via the oral cavity due to translocation of the bacteria into the gastrointestinal tract [10]
E.faecalis is a gram-positive bacterium, this bacterium can cause endocarditis, is resistant to many antibiotic agents and can bind to host cells. It also has many survival mechanisms to live in unfavourable conditions such as low oxygen, high pH or nutrient poor environment.[3]

The factors most extensively studied are aggregation substance, surface adhesins, sex pheromones, lipoteichoic acid, extracellular superoxide, gelatinase, hyaluronidase, and cytolysin. A relationship was observed between percussion tenderness and *E.faecium* in the study of Felipe Paiva Machado and al.[9]

F.nucleatum is a gram-negative bacteria able to bind, invade and survive inside human cells via the adhesin FadA, also influence the formation and progression of periapical lesions through the Wnt/β-catenin pathway. It is considered anaerobic invasive, adherent, and inflammatory.[10]

T.denticola have a lot of virulence factors like motility, low immunogenicity, invasion of host tissues and secretion of proteolytic enzymes that enable them to rapidly colonise locations, penetrate tissues and avoid the host defence systems.[3]

Endotoxins are toxins located in the outer membrane of certain Gram-negative bacteria, lipopolysaccharide (LPS) in nature and thermostable. Lipoteichoic acid (LPA) is a major constituent of the cell wall of gram-positive bacteria. In the different studies, we observed that specific microorganisms were associated with symptoms and signs and LPS was positively correlated with larger periapical bone destruction. The presence of bacteria and their virulence factors in periapical lesions may delay the healing of lesions after root canal treatment, the characterisation of these microbial virulence factors is aimed at and helps to clarify the pathogenesis of the infection.[9,10]

STUDY	TECHNIQUE USED	HIGH PREVALENCE BACTERIA (>50%)	PREVALENCE	VIRULANCE FACTOR and PATHOGENICITY
Felipe Paiva Machado and al.[9]	Samples collected with paper points in culture identified with checkerboard DNA-DNA hybridization.	*P.gingivalis* (Gram -)	72.41%	Pathological bone loss Inflammation Fimbriae
		E. faecalis, S. constellatus, E. faecium (Gram +)	58.62%	resistant to many antibiotic agents inflammation endocardite fixation host cells
		F.nucleatum and *E.nodatum* (Gram -)	65.62%	fadA adhesin (invade and survive inside human cells) regulate the Wnt/β-catenin pathway. periodontitis
		C.rectus (Gram -)	68.97%	bone loss chronic periodontits
		S.mitis (Gram +)	62.07%	bactériémies endocardite adhesion to fibronectin

J. Wang and al.[15]	Apical root samples from root-end surgery on root-filled teeth with persistant AP.	*Actinomyces sp.* (Gram +)	84.6%	structure fimbrial osteonecrosis subacute or chronic indurated abscesses stimulation of the immune system
		Propionibacterium (Gram +)	61.5%	structure fimbrial allows growth of vitamin K bacteria
J. D. Bronzato and al.[10]	Samples from endodontic microsurgery and Polymerase Chain Reaction	*P.micra* (Gram +)	87.5%	adhesins (host cell adhesion) proteases (host invasion) capsule periodontitis, gingivitis, abscesses
		E.faecalis (Gram +)	75%	resistant to many antibiotic agents inflammation endocardite fixation host cells
		F.nucleatum (Gram -)	68%	FadA adhesin (invade and survive inside human cells) regulate the Wnt/β-catenin pathway. periodontitis
Victor DJ and al.[3]	Samples collected with paper point and RT-PCR technique	*E.faecalis* (Gram +)	92%	resistant to many antibiotic agents inflammation endocardite fixation host cells

		P.intermedia and *P.gingivalis* (Gram -)	56%	Pathological bone loss Inflammation Fimbriae
		T.denticola (Gram -)	86%	adherence cytotoxicity proteolytic enzymes
Pereira R.S. and al.[6]	RT-PCR technique	*F.nucleatum* (Gram -)	71.6%	FadA adhesin (invade and survive inside human cells) regulate the Wnt/β-catenin pathway.
		D.pneumosintes (Gram -)	58.3%	gingivitis periodontitis sinusitis

Table 2: Relevant data from studies about prevalence of bacteria

5.3.2 Measurements and volumes

Presentation and classification of the different periapical lesions:

- Acute apical periodontitis is an inflammation that is classified as primary if it is a short-lived inflammation initiated on a healthy apex. When pathogenic bacteria are involved, this response may progress to abscess. It will be termed secondary if it occurs on an already existing chronic periodontitis, usually in the form of an abscess. [23]
- Chronic apical periodontitis, also known as apical granuloma, is an inflammation evolving over a long period of time, characterised by the presence of granulation tissue infiltrated by lymphocytes, plasma cells and macrophages.[23]
- The periapical cyst is an inflammatory cyst with a distinct epithelium delimiting a cavity. It is called a true cyst if the cavity lined by the epithelium is completely closed so that it does not communicate with the canal. It is called a pocket or bay cyst if the cyst is presented in the form of a sac, so that that the epithelial envelope bordering the cavity is open and continuous with the with the root canal.[23]

Modern imaging technology allows for the diagnosis of endodontic disease and the use of endodontic treatment to be simplified. CBCT methods represent an improvement over standard two-dimensional radiographs (panoramic and periapical) and accurately measure the volume of periapical lesions. This allows an assessment of the dynamics of the lesion, whether it is recovering, asymptomatic or deteriorating. CBCT is used in dental clinics for its high quality, specificity and ability to produce 3D images.[13,17]

CBCT imaging requires precise settings, *Felipe Paiva Machado and al.* used classic I-cat with 8 cm field of view, 120 kVp, 36.15 mA and 12-bit depth. All images were converted to digital format (DICOM) and evaluated with NEMOTEC software. After determining the measurements at the 3 planes, a polygon delimiting the lesion was created to proceed with the 3D reconstruction of the periapical radiolucency at a threshold of 350 Hounsfield units. The median volume of bone destruction determined by CBCT imaging in PAP and PTAP was 60 mm3 (20-280 mm3) and 60 mm3 (10-680 mm3) respectively. Although the CBCT data

showed similarity in both infections, with no statistical difference, PTAP had higher lesion volume values than PAP.[9]

In the study by *Kamburoğlu K and al*., CBCT images were obtained using a CMOS flat panel detector with a small FOV (50 mm 50 mm) Kodak CS 9300 3D (Carestream Health Co, Rochester, NY). The settings were set at 80 kVp, 8mA, 12 seconds and a voxel size of 0.09 mm with an area dose product value of 628 mGy.cm2. They found that the volume and surface area of the periapical lesions before treatment varied considerably from case to case; their volume ranged from 12.77 mm3 to 1080.89 mm3 and their surface area from 431.17 mm2 to 2014.11 mm2, and the periapical lesion volume and area after treatment ranged from 2.31 mm3 to 168.29 mm3 and from 6.43 mm2 to 358.85 mm2 in area. We also found that the maximum sinus mucosa thickness before treatment ranged from 2 mm to 12.200 mm and the maximum sinus mucosa thickness after treatment ranged from 1 mm to 7 mm. In addition, regression analysis of pre-treatment lesion volume against post-treatment volume change and sinus thickening measurements revealed a linear relationship, suggesting that there was no correlation between lesion volume and lesion volume reduction, sinus measurements and sinus thickening change.[12]

We can observe reduction in periapical lesion height, surface area and volume by using CBCT in lesions one year after the treatment. We also observe a large variety of measurements depending on the lesions, and no correlation between the volume of the lesion and the volume reduction on time.[12,13]

5.3.3 Progress and Dynamism

The prevalence of PA increases with age and if a tooth has already undergone endodontic treatment. The extent of a lesion depends on the number of bacteria present in the root canal, and the dynamism and evolution of a lesion are closely related to the microorganisms present.[16,17]

In the study by *Sisko Huumonen & Dag Orstavik and al.,* observed that regardless of the size of the lesion, in most cases the lesion size decreased by up to 2 mm within 2 years after

endodontic treatment. Sometimes the size decrease can continue from 4 to 9 years. In their studies, they observed that maxillary canines had the highest incidence of periodontal disease, while upper central incisors developed no AP. Among the different types of teeth, the upper lateral incisors had the lowest healing rate, and the mandibular premolars were the teeth with the highest percentage of healing.[16]

In the study by *Victoria Soo Hoon Yu and al*., Of the 151 lesions studied, 86.6% showed improvement, 11.9% remained the same and 31.1% deteriorated. A decade after treatment, most lesions had decreased in size. An ideal root filling length (approximately 2 mm from the apex) was found in 78.2% of cases, with adequate density in 86.1% and adequate coronal restoration in 74.2%. Iatrogenic errors such as the presence of intracanal space between post and gutta percha, instrument separations and perforations were identified in 15.3% of cases. Root filling length showed a relationship with lesion progression, the other variables were irrelevant in this point. Many small lesions (diameter <5 mm on radiograph) decreased in size over the observation period, while larger lesions (diameter > or =5 mm) mostly reflected an increase in size over time. It has been observed that complete resolution of a lesion can occur even over long periods of 10 to 20 years after endodontic treatment.[11]

In the study by *Kamburoğlu K et al.,* there was 36% mean reduction in lesion width, 41% in lesion height, 53% in maximum sinusal mucosal thickening, 54% in average sinusal mucosal thickening, 53% in minimum sinusal mucosal thickening, 73% in lesion area and 75% in lesion volume. These results show significant reduction in periapical lesion width, lesion height, surface area and thickening of the adjacent sinus mucosa using CBCT and specific software 1 year after endodontic treatment. No significant differences were found for variables such as gender, age and root type.[12]

In the study by *Igor Tsesis and al.*, the correlation between the periapical lesion and bacterial infections is verified, so AP does not heal without intervention. The results of the progression of lesions are as follows: fifty-seven (28.5%) lesions remained unchanged, 103 (51.5%) lesions worsened (increase in PAI score) and 40 (20%) lesions improved. They used the PAI score which consists of 5 categories, each representing a step on an ordinal scale

from normal periapical bone to severe PA. It was found that in some cases, recovery from PA after an RCT took 4 years to complete.[14]

In the study by *Ming-Ming Zhang and al.,* the 13 lesions which had completely disappeared at the 2-year follow-up visit had already become small at the 1-year follow-up visit, suggesting that the greatest reduction in size occurred in the first year. For the other periapical radiolucencies, which decreased even in the second year, reduction speed was slower in the second year than in the first year after treatment. This result may indicate that the initial "healing process" is most effective. [13]

Several factors are important in the progression of the lesions, the quality of the filling especially about the length of the root filling, the bacterial flora and the size of the lesion. Other variables such as shape, edge, instrumental incident during treatment and perforation are negligible and don't give more information about lesions progression. Flare-ups, persistent symptoms, and bite pain are strongly associated with deteriorating lesions but are uncommon and most lesions remain asymptomatic after endodontic treatment. The duration of lesion recovery is uncertain but the first year after treatment is the year in which lesion healing is most significant. Smaller lesions are more likely to heal completely which shows the importance of early management of a periapical lesion.[11,13,14]

5.4 Possible Therapies

The treatment options for apical periodontitis for teeth that have already undergone endodontic treatment are broader than for a tooth that has not undergone any previous treatment. The most important aspect of recovery from an apical lesion is its follow-up, and it may be more appropriate to use the term management rather than cure.[13,15] During follow-up in cases of persistent AP, further endodontic treatment or extraction of the tooth are usually the predominant choices. If the lesion is asymptomatic, a longer-term follow-up is considered as the management of the lesion may be successful if the symptoms and progression of the lesion have resolved.[11,13,14] Check-ups are recommended until the periapical pathologies have completely disappeared.[16]

For the follow-up of lesions, the use of cone beam tomography (CBCT) allows, in addition to an effective radiation dose and a lower cost, the acquisition of images of higher definition than medical tomography. CBCT can detect periapical pathology before it is apparent on the periapical radiograph, with the advantage of a three-dimensional assessment.[12] In the clinic, practitioners and patients often must decide about which procedure to perform. Sometimes the decision to extract is made without access to good radiology, whereas a radiological assessment of the lesion dimensions can provide useful information for diagnosis, differentiation, treatment plan and review of periapical disease.[11,12] More in-depth studies on periapical lesions would help us to improve diagnosis and treatment choices.

5.5 Limits

5.5.1 Limits of the studies

The subject of this study is vast, which forced us to restrict the information given and to do a lot of research in parallel with the observed studies.

The data obtained in the studies observed in this systematic review may be slightly distorted and different from other similar studies due to: [3,9,11,12,15,16]

- Instrument use and removal of root filling material may induce microorganism removal in PTAP lesions.
- The microbiological culture technique only detects viable and cultivable bacteria, sometimes underestimating the presence of critical species that cannot be cultivated. The culture environment used, and the transport time of the samples influence the presence of certain species.
- The radiographic aspect has its limits in the differential diagnosis of periapical lesions and its reduction
- Incorrect use of software and misinterpretation of CBCT images that may occur due to inexperience
- The results of each study are specific to the technique used
- The results of each study are specific to the technique used
- Differences in sample collection, methodology and clinical conditions of patients
- Substantial reduction in teeth at recall appointments, may be a bias due to dropouts

5.5.2 Limits of current science

Two-dimensional intraoral periapical radiography provides only limited information regarding the origin, size and location of periapical lesions and is unable to detect the early stages of periapical lesions. The presence of more pronounced scatter compared to medical CT and metal artefacts may limit CBCT imaging. These artefacts can be caused by metal or amalgam restorations and sometimes by filling materials and implants.[12,17]

Sample collection does not always reach bacteria located in remote sites in the canal, in dental tubules, lateral canals, apical branches and isthmuses. Collection techniques must be thorough to avoid underestimation of the number of microorganisms. Samples may also be contaminated with saliva, other fluids and blood. Although there have been technological advances in materials, equipment, automated preparation techniques, apical locators, surgical microscopes, CBCT for diagnosis, observation and culture techniques, and dissemination of knowledge in an accessible manner, the results of the various studies indicate that there is a long way to go before total success and total knowledge about endodontic treatment and periapical lesions. [6,9,10,15]

6 CONCLUSION

With the development of current science, we can increase our knowledge of periapical lesions. In particularly their bacterial flora and their dynamism, which could allow us to predict a periapical lesion and to facilitate treatments by improving their chances of success. In this study, we noted an improvement in post-treatment lesions which proves the effectiveness, although not absolute, of endodontic treatment.

This review gives a global overview of lesions so more precise studies are needed to refine our knowledge of periapical diseases.

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