



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de São José dos Campos
Instituto de Ciência e Tecnologia

DIEGO GUILHERME DIAS DE RABELLO

**ENDOTOXINAS NAS INFECÇÕES ENDODÔNTICAS: revisão
sistemática com metanálise e ensaio clínico randomizado**

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sistemática com metanálise e ensaio clínico randomizado**

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BANCA EXAMINADORA

Prof. Adj. Frederico Canato Martinho (Orientador)

Universidade Estadual Paulista (Unesp)

Instituto de Ciência e Tecnologia

Campus de São José dos Campos

Prof. Aletéia Massula de Melo Fernandes

Faculdade Anhanguera

Campus de São José dos Campos

Prof. Adj. Ana Paula Martins Gomes

Universidade Estadual Paulista (Unesp)

Instituto de Ciência e Tecnologia

Campus de São José dos Campos

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“Senhor, dai-me força para mudar o que pode ser mudado...
Resignação para aceitar o que não pode ser mudado...
E sabedoria para distinguir uma coisa da outra”.
(São Francisco de Assis)

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RESUMO

Sabe-se que as endotoxinas provenientes das bactérias grã-negativas desempenham um importante papel nas infecções endodônticas se relacionando à sinais e sintomas clínicos/radiográficos. Sendo assim, a redução ou eliminação de endotoxinas é fundamental para a resolução da inflamação periapical. Os objetivos do presente estudo foram: **Artigo 1** - realizar uma revisão sistemática com metanálise com o intuito de avaliar a relação entre níveis de endotoxinas e a presença de sinais e sintomas clínicos, bem como de sinais radiográficos em pacientes com infecção endodôntica primária; **Artigo 2** - fazer um ensaio clínico randomizado para avaliarmos estratégias clínicas para otimizar a eliminação microbiana e de lipopolissacarídeos (LPS) utilizando a Terapia Fotodinâmica (PDT) como tratamento suplementar ao PQM nas modalidades de terapia endodôntica em única e múltiplas sessões. Métodos: **Artigo 1** - para a revisão sistemática uma busca eletrônica foi realizada por dois autores, no idioma inglês, nas bases de dados Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge e Scopus. Após seleção dos artigos foi realizado uma metanálise com análises de sinais e sintomas clínicos/radiográficos. **Artigo 2** - Selecionou-se 24 casos de dentes com infecção endodôntica primária que foram aleatoriamente divididos em 2 grupos (n=12): SU - sessão única e SM – sessão múltipla. Foram realizadas coletas microbiológica e de endotoxinas dos canais radiculares utilizando cone de papel estéril/ apirogênico em diferentes momentos operatórios. Foi realizado cultura microbiana para determinar a carga bacteriana em UFC/mL (Unidades Formadoras de Colônias/mL) e para quantificação de endotoxinas foi realizado o teste KQCL de “Lisado de Amebócito Limulus” (LAL). Resultados: **Artigo 1** - A metanálise revelou que indivíduos com dor à percussão (TTP) ($P=0,04$; I^2 57%) e episódio de dor prévio (PEP) ($P=0,001$; I^2 81%) apresentaram maior nível de endotoxina que os casos que não apresentaram nenhum destes sintomas. O tamanho da área radiolúcida (SRL) > 2 mm ($P=0,02$; I^2 68%) e presença de exudação (EX) ($P=0,0007$; I^2 0%) estavam associados com maior nível de endotoxinas. **Artigo 2** - Para carga microbiana, a suplementação do tratamento em sessão única com PDT reduziu significativamente os níveis bacterianos ($p<0,05$), mas não nos casos de duas sessões ($p>0,05$). Para os níveis de endotoxinas, na sessão única e de duas sessões, não houve diferença significativa após uso de PDT ($p>0,05$). Foi possível concluir que **Artigo 1** – A metanálise mostrou forte evidência que endotoxina está relacionada com a presença de sinais e sintomas clínicos/radiográficos em pacientes com infecção endodôntica. **Artigo 2** - A terapia fotodinâmica melhorou o desempenho da desinfecção bacteriana dos canais radiculares em uma visita, mas não para a modalidade de 2 visitas após uso de medicação à base de Ca(OH)_2 . PDT não foi efetivo contra endotoxinas.

Palavras-chave: Periodontite periapical. Tratamento do canal radicular. Desinfecção. Endotoxina. Revisão sistemática. Metanálise.

Rabello DGD. *Endotoxins in endodontic infection: a systematic review with meta-analysis and a clinical trial [dissertation]*. São José dos Campos (SP): Sao Paulo State University (Unesp), Institute of Science and Technology; 2016.

ABSTRACT

Endotoxins originating from gram negatives bacterias are related to signals and clinical/radiographic symptoms. Therefore, reduction or elimination of endotoxins is essential to solve periapical inflammation. The aim of this dissertation was: **Article 1** - To relate endotoxin levels and presence of clinical signs/symptoms and radiographic features in endodontic infection. **Article 2** - To evaluate the effectiveness of supplemental photodynamic therapy (PDT) in optimizing the removal of bacteria and endotoxins from primarily infected root canals after one-visit and two-visit treatments. Methodology: **Article 1** - Electronic searches were performed on Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge and Scopus databases for identification of relevant studies published up to July 2016. Only reports in English were included. The selected literature was reviewed by two authors and classified as either suitable or unsuitable for inclusion in this review. The relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features were determined. Additionally, a meta-analysis was performed. **Article 2** - 24 primarily infected root canals with apical periodontitis were selected and randomly divided into one-visit (n=12) and two-visit (n=12). Samples were collected before and after root canal procedures. Endotoxins were quantified by chromogenic limulus amebocyte lysate assay. Culture techniques were used to determine bacterial colony-forming unit counts. The systematic review and meta-analysis aimed to evaluate the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection. Results: **Article 1** - Among the 285 articles identified in the initial search, 29 were included for full-text appraisal and only eight studies met the inclusion criteria for this systematic review. Meta-analysis revealed that individuals having teeth with tenderness to percussion (TTP) ($P=0.04$; I^2 57%) and previous episode of pain (PEP) ($P=0.001$; I^2 81%) had higher levels of endotoxin than their counterparts. Size of radiographic lesion (SRL) > 2 mm ($P=0.02$; I^2 68%) and presence of root canal exudation (EX) ($P=0.0007$; I^2 0%) were associated with higher levels of endotoxin. **Article 2** - Bacteria and endotoxins were detected in 100% of the initial samples, with median values of 1.97×10^5 CFU/mL and 24.983 EU/mL, respectively. The CMP using single-file reciprocating technique was effective in the reduction of bacteria and endotoxins (All, $p < .05$). The supplemental PDT was effective in reducing bacterial load in the one-visit ($p < .05$) but not in the two-visit treatment after use of Ca(OH)_2 medication for 14 days ($p > .05$). In the two-visit group, after 14 days of inter- appointment medication with Ca(OH)_2 , a significant reduction in the median levels of endotoxins was found in comparison to CMP alone (from 1.041 to 0.094 EU/mL) ($p < .05$). Despite the type of treatment, the supplemental PDT was not effective against endotoxins ($p > .05$). Conclusion: **Article 1** - This meta-analysis provides strong evidence that endotoxin are related with the presence of clinical signs/symptoms and radiographic features in patients with endodontic infection. **Article 2** - The photodynamic therapy optimized the disinfection of bacteria from root

canals in one-visit but not for two visit treatment modality with the accomplishment of calcium hydroxide medication. Thus, PDT is not effective against endotoxins.

Keywords: Bacterial reduction. Disinfection. Endodontic treatment. PDT. Photodynamic therapy. Endotoxins. Systematic review. Meta-analysis.

1 INTRODUÇÃO

Microrganismos e seus subprodutos atuam como fatores de desenvolvimento e perpetuação das lesões pulpares e perirradiculares (Takehashi *et al.*, 1965).

A maioria das bactérias capazes de infectar os canais radiculares é oriunda da cavidade oral (Loesche, 1976). Entretanto o ambiente seletivo do canal radicular faz com que apenas alguns microrganismos sejam capazes de colonizar este sistema. Os microrganismos residentes do canal radicular são selecionados através de fatores locais e sistêmicos tais quais : temperatura, pH, acesso à nutrientes, capacidade de defesa do hospedeiro, tensão de oxigênio (Sundqvist, 1992).

As infecções endodônticas primárias polimicrobianas são dominadas por bacilos Gram-negativos anaeróbios estritos (Fabricius *et al.*, 1982) que possuem na composição da membrana externa, os lipopolissacarídeos (LPS), também conhecidos como endotoxinas, que podem ser liberados nos processos de multiplicação ou morte bacteriana (Gomes *et al.*, 1994; Martinho *et al.*, 2010). O lipídio A é o componente bioativo do LPS responsável pela maioria da respostas imunes.

Uma alta concentração de endotoxinas têm sido associada à maior destruição óssea em casos de infecção endodôntica (Horiba *et al.*, 1991) Endotoxinas estimulam reabsorção óssea em cultura de células (Norton *et al.*, 1970; Hong *et al.*, 2004; Bandow *et al.*, 2010; Kassem *et al.*, 2015).

Alguns estudos clínicos detectaram a presença de LPS bacteriano em canais radiculares de dentes com infecções endodônticas primárias e lesão periapical indicando sua possível participação numa maior destruição óssea perirradicular (Martinho *et al.*, 2012, 2014; Xavier *et al.*, 2013; Cardoso *et al.*, 2016).

Existem trabalhos clínicos que mostram a associação da concentração de endotoxinas com sinais e sintomas clínicos relacionados à dor tais quais dor espontânea, dor à percussão, dor à palpação. (Schein, Schilder, 1975; Martinho, Gomes, 2008; Martinho *et al.*, 2010, 2011; Gomes *et al.*, 2012, Herrera *et al.*, 2016). Além do mais, a penetração através do foramen apical pode perpetuar periodontite apical (Horiba *et al.*, 1991; Vianna *et al.*, 2007).

O acúmulo de componentes bacterianos em área infectada, particularmente endotoxinas (incluindo ácido lipoteicoico, peptidoglicanas, lipopolissacarídeos) podem estimular o lançamento de citocinas pró-inflamatórias por diferentes vias tais quais ativação de TLR – 2, TLR- 4. (Hong *et al.* 2004; Horiba *et al.*, 1989). O tecido inflamatório presente nas lesões perirradiculares é ocupada predominantemente por macrófagos (Hosoya, Matsushima, 1997; Matsuo *et al.*, 1992) que são as maiores fontes de IL -1 β (Stashenko *et al.*, 1987) e produtor quase exclusivo do TNF- α (Beutler, Cerami,1986) quando na presença de bactérias e/ou LPS.

Endotoxinas são potentes agentes inflamatórios com vias de ativação clássica e do sistema complemento. (Morrison, Kline, 1977; Seltzer, Farber, 1994). O sistema complemento libera peptídeos biologicamente ativos, que liberam um número de aspectos do processo inflamatório (Morrison, Kline, 1977). LPS pode provocar dor através da ativação do fator Hageman ou através propriedades neurotóxicas quando ativam terminos dos nervos pré-sinápticos (Ferraz *et al.*, 2015).

Uma rede mais complexa entre diferentes mediadores inflamatórios parecem implicar em desenvolvimento de sinais e sintomas clínicos e radiográficos (Martinho *et al.*, 2012).

Devido à estas evidências científicas, foi realizado uma revisão sistemática com metanálise com o intuito de avaliar a relação entre níveis de endotoxinas e a presença de sinais e sintomas clínicos, bem como de sinais radiográficos em pacientes com infecção endodôntica.

Sabe-se que uma desinfecção do sistemas de canais radiculares efetiva consiste não apenas na eliminação de bactérias, de restos pulpares, mas também desbridamento e eliminação de biofilmes (Clarkson *et al.*, 2012; Guneser *et al.*, 2015; Case *et al.*, 2012) assim como eliminação e neutralização de fatores de virulência (Martinho, Gomes, 2008; Xavier *et al.*, 2013).

Apesar dos avanços nas técnicas de instrumentação com uso de limas reciprocantes (Martinho *et al.*, 2010, 2014, Neves *et al.* 2016) ou de limas rotatórias NiTi em rotação contínua (Cardoso *et al.*, 2015; Neves *et al.*, 2016), estudos que preconizaram sessão única estão falhando em demonstrar uma desinfecção completa do sistemas de canais (Martinho *et al.*, 2010, 2014; Xavier *et al.*, 2013; Vera *et al.*, 2012; Ferreira *et al.*, 2015).

Na busca por métodos suplementares para otimizar a desinfecção do canal radicular, a terapia fotodinâmica (PDT) tem sido considerado recentemente (Bonsor *et al.* 2006, Garcez *et al.*, 2008, 2010; Fimple *et al.*, 2008; Soukos *et al.*, 2006; Cherpa *et al.*, 2014). Contudo, poucos estudos clínicos avaliaram a efetividade do PDT na desinfecção do sistema de canais radiculares (Bonsor *et al.*, 2006; Garcez *et al.*, 2008, 2010). Além disto, não há estudos clínicos avaliando a efetividade do PDT na redução e/ou eliminação de endotoxinas dos canais radiculares com lesão periapical primária.

Um dos motivos para a utilização do PDT é o alto índice atual da resistência bacteriana aos antibióticos que acabou gerando maior interesse em terapias antimicrobianas alternativas (Karkwal *et al.*, 2011). O PDT, por sua ação antimicrobiana, pode representar uma opção de tratamento no controle de patógenos resistentes, e pode vir a ser uma opção como possível abordagem para tratar infecções multirresistentes (Oliveira *et al.*, 2015). No entanto, a sua efetividade é dependente de alguns fatores, tais quais: dose, tempo de incubação, local em que será aplicado o agente de fotossensibilização, disponibilidade de oxigênio no local, comprimento de onda utilizado, densidade da potência da luz e fluência de energia da luz (Chrepa *et al.*, 2014).

A fim de elucidar o papel das endotoxinas nas infecções endodônticas primárias, este estudo buscou avaliar a efetividade da suplementação da terapia fotodinâmica (PDT) em otimizar a remoção bacteriana e de endotoxinas dos canais de infecções endodônticas primárias após tratamento em uma e duas visitas.

2 ARTIGOS

2.1 Artigo – Martinho FC, Rabello DGD, Ferreira LL, Nascimento GG. Endotoxina em infecção canal radicular: uma revisão sistemática e metanálise / *Endotoxin in root canal infection: a systematic review and meta-analysis* *

RESUMO

Foi realizado uma revisão sistemática com metanálise com o intuito de avaliar a relação entre níveis de endotoxinas e a presença de sinais e sintomas clínicos, bem como de sinais radiográficos em pacientes com infecção endodôntica. Para isto uma busca eletrônica foi realizada por dois autores, no idioma inglês, nas bases de dados Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge e Scopus. Entre 285 artigos identificados, 29 foram incluídos para leitura completa e 8 artigos foram incluídos por estarem dentro dos critérios de inclusão. A metanálise revelou que indivíduos com dor à percussão (TTP) ($P=0,04$; I^2 57%) e episódio de dor prévio (PEP) ($P=0,001$; I^2 81%) apresentaram maior nível de endotoxina que os casos que não apresentaram nenhum destes sintomas. O tamanho da área radiolúcida (SRL) > 2 mm ($P=0,02$; I^2 68%) e presença de exudação (EX) ($P=0,0007$; I^2 0%) estavam associados com maior nível de endotoxinas. Esta metanálise mostra evidência de que endotoxina está relacionado com presença de sintomas e sinais clínicos e radiográficos em pacientes com infecção endodôntica.

Palavras-chave: Periodontite periapical. Tratamento do canal radicular. Endotoxina. Revisão sistemática. Metanálise.

ABSTRACT

Introduction: This systematic review and meta-analysis aimed to evaluate the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection. Methods: Electronic searches were performed on Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge and Scopus databases for identification of relevant studies published up to July 2016. Only reports in English were included. The selected literature was reviewed by two authors and classified as either suitable or unsuitable for inclusion in this review. The relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features were determined. Additionally, a meta-analysis was performed. Results: Among the 285 articles identified in the initial search, 29 were included for full-text appraisal and only eight studies met the inclusion criteria for this systematic review. Meta-analysis revealed that individuals having teeth with tenderness to percussion (TTP) ($P=0.04$; I^2 57%) and previous episode of pain (PEP) ($P=0.001$; I^2 81%) had higher levels of endotoxin than their counterparts. Size of radiolucent area (SRL) > 2 mm ($P=0.02$; I^2 68%) and presence of root canal exudation (EX) ($P=0.0007$; I^2 0%) were associated with higher levels of endotoxin. Conclusion: This meta-analysis provides strong evidence that endotoxin are related with the presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

Keywords: Endodontic treatment. Endotoxins. Systematic review. Meta-analysis.

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ENDOTOXIN IN ROOT CANAL INFECTION: A SYSTEMATIC REVIEW AND META-ANALYSIS

INTRODUCTION

A great diversity of Gram-negative bacteria has been identified in root canal infection (1-5), especially species belonging to Prevotella spp., Porphyromonas spp. and Fusobacterium spp (6, 7). The cell wall of Gram-negative bacteria contains lipopolysaccharide (LPS), a complex molecule, which is generally referred to as endotoxin (8).

Clinical studies have detected endotoxins in infected root canals (1-3,9-18), and most of them have suggested a correlation between endotoxin levels and development of clinical symptoms whereas others have failed (4,9,12,13,15,19-22). Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of complement system (23,24). Complement activation releases biologically active peptides, which mediate a number of aspects of the inflammatory process (23). LPS may evoke pain through activation of the Hageman factor or through neurotoxic properties when acting on presynaptic nerve terminals (24), direct sensitization of nociceptors (25), sensitization and up-regulation of the transient receptor potential cation channel, sub-family V, member 1 (TRPV1) (26,27).

Endotoxins have been found to stimulate stimulation of bone resorption in tissue culture (28-31). Some clinical studies have demonstrated a positive correlation between higher levels of endotoxin in infected root canals and larger area of periapical bone destruction, whereas others have failed (4,18,20,32). A more complex network between different inflammatory mediators seems to be implicated in the development of clinical symptoms and radiographic features (32). The LPS released from infected root canal triggers the synthesis of IL-1 and TNF-alpha (30, 33), which in turn up-regulates the production of MMP-1 by macrophages to promote periapical bone resorption (30). Furthermore, LPS stimulates bone resorption by enhancing RANKL (receptor activator of NF-kB ligand) through activation of toll-like receptor-2 in osteoblasts (31) as well as inhibitory effect on osteoblast differentiation (29).

It is important to highlight that an overall analysis of data from studies correlating presence of endotoxins with clinical and radiographic features has not

been performed yet. One of the main advantages of a systematic review with a statistical approach, such as meta-analysis, is to address sources of bias in order to produce the most valid and precise estimate of effect as possible (34). Therefore, the main purpose of this study was to conduct an extensive systematic review of the literature on endotoxin in endodontic infections in order to elucidate the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

MATERIAL AND METHODS

Review Question

Is there a relationship between endotoxin levels and presence of clinical/radiographic symptoms in patients with periapical periodontitis?

Inclusion and Exclusion Criteria

Studies were included based on the following inclusion criteria:

- 1. Clinical studies in humans;*
- 2. Endodontic infection must be present;*
- 3. Quantification of endotoxin and description of the test;*
- 4. Evaluation of clinical/radiographic symptoms;*
- 5. Publication in English.*

Studies were eliminated if the inclusion criteria were not met or if they presented any of the following exclusion criteria:

- 1. In vitro or animal study, case report, review article, or opinion article;*
- 2. Lack of mean level of endotoxin and standard deviation, or data that allow their calculation;*
- 3. Lack of evaluation of the endotoxin levels;*
- 4. Lack of description of the type of endodontic infection;*
- 5. The publication was based on a population from another study;*
- 6. Study evaluating endotoxin in primary teeth;*

7. *The same levels of endotoxins described by an author in more than one study;*
8. *Lack of clear definition of the method used for endotoxin detection.*

Search Strategy

The literature was searched to identify published articles analysing the presence of endotoxin in endodontic infection. Electronic searches were performed on Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge and Scopus databases for identification of relevant studies published up to July 2016. An initial search was conducted on PubMed by using medical subject heading (MeSH) terms and other free terms as follows: “periapical periodontitis, OR periapical diseases, OR dental pulp diseases, OR apical periodontitis, OR endodontic infection AND endotoxin, OR LPS”. No language restrictions were applied within the search.

All references were tabulated by using the software EndNote X6. Duplicate references were excluded. Titles, abstracts and key-words were screened by two reviewers (DDR and LLF) based on inclusion and exclusion criteria. The cases of disagreement between authors were solved after discussion. After initial screening of titles and abstract, the full articles were evaluated by the same two reviewers. In addition to the electronic search, a hand search of the reference lists of the selected articles was performed. Pre-defined data collection worksheets were used for assessment of each selected publication.

Data Extraction

Relevant data were extracted from the selected articles based on study description (i.e. setting, sample), endotoxin detection method, type of endodontic infection (i.e. primary/secondary) and presence of clinical or radiographic feature. Furthermore, in order to conduct the meta-analysis, the mean level and standard deviation of endotoxins were gathered. The authors were contacted when further clarifications regarding the study methodology or results were required. Data were extracted by two reviewers (DDR and LLF) independently by using pre-piloted data extraction forms. In case of disagreement, discussions were held to resolve and reach consensus. All the stages of this systematic review were supervised by a third

reviewer (GGN) who has expertise in systematic review methodology.

Quality assessment

The Critical Appraisal Checklist recommended by JBI (34) was adapted for quality assessment. The checklist was composed of 8 items as indicated in Table 1. Reviewers should answer with 'Yes', 'No' or 'Unclear' for each item. To classify studies by quality, an overall score for each study was calculated based on the number of 'Yes' answers. Thus, scores could range from 0 to 8. Finally, studies were categorised in tertiles according to the score obtained, as follows: 1st tertile (0–2): low quality; 2nd tertile (3–5): medium quality; and 3rd tertile (6–8): high quality. Studies were assessed independently by two reviewers, and disagreements were resolved by consensus. The quality assessment is provided in table 1.

Statistical Analysis

Five different meta-analyses were conducted in order to evaluate the levels of endotoxin in the presence of each clinical symptom as follows: pain on palpation (POP), tenderness to percussion (TTP), previous episode of pain (PEP), exudation (EX) and radiographic lesion greater than 2 mm (SRL). For each model, the standard mean difference was obtained with fixed- and random-effect models. If heterogeneity was present ($P < 0.05$; $I^2 \geq 50\%$), the random-effect model was employed (35). Median and range values were converted into mean and standard deviation according to Hozo et al. (36). The analyses were conducted by using the software RevMan 5.3 (The Nordic Cochrane Center, Copenhagen, Denmark, 2014).

RESULTS

Electronic search revealed 285 articles. After removing 41 duplicate articles, 244 articles were included for title and abstract screening. Twenty-nine articles were included for full-text appraisal, including one article identified in the reference list. Twenty-one articles were excluded after full-text assessment. Subsequently, eight studies met the inclusion criteria in this systematic review. Figure 1 displays the flowchart of the study selection. The samples in the included studies totaled 231 individuals. Six studies were conducted in Brazil (1,3,9,11-13), one in Japan (20) and

one in the United States (19) (Table 2). With regard to the methods used to quantify endotoxins, two studies used Gel Clot LAL assay (19,20), two used Endpoint Chromogenic LAL assay (9,13), three used Kinetic Turbidimetric LAL assay (1,11,12) and one compared all these three tests (2) (Table 2). Meta-analysis revealed that individuals with tenderness to percussion (TTP) ($P=0.04$; I^2 57%) and previous episode of pain (PEP) ($P=0.001$; I^2 81%) presented higher levels of endotoxin than their counterparts (Figure 2). No association between endotoxin concentration and pain on palpation (POP) was noted ($P=0.87$; I^2 45%). A higher level of endotoxins was associated with the presence of exudation in infected root canals (EX) ($P=0.0007$; I^2 0%) and larger size of radiolucent area (SRL >2mm) ($P=0.02$; I^2 68%). Meta-analysis and graph for symptoms and radiographic features are shown in Figure 2.

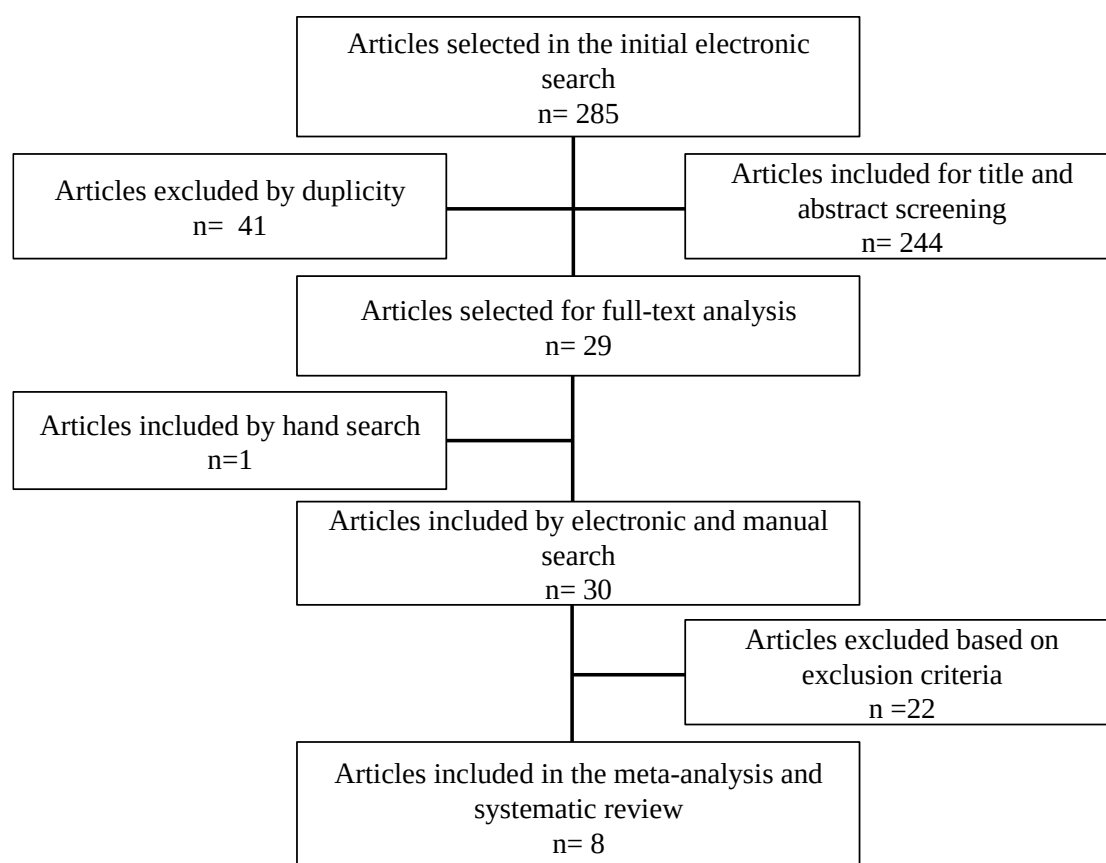


Figure 1. Flowchart of the articles screened in the review process.

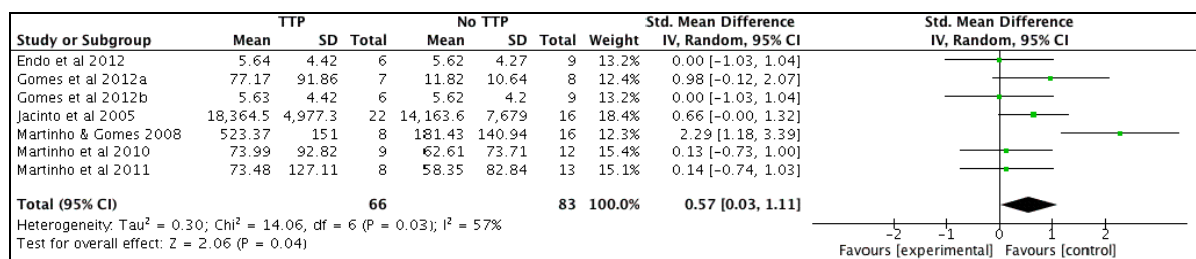
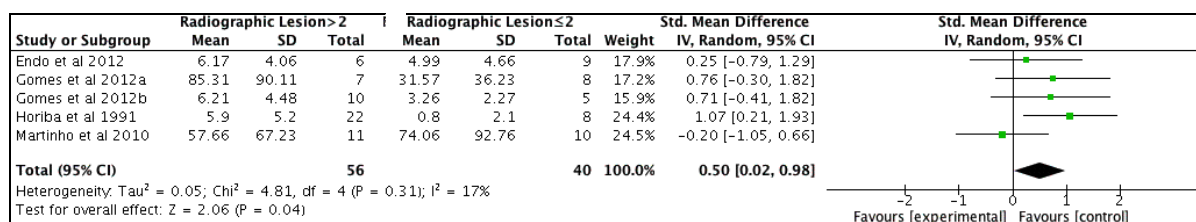
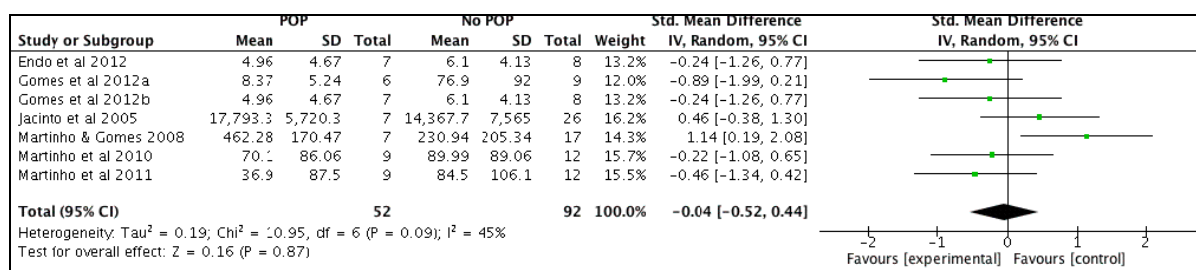
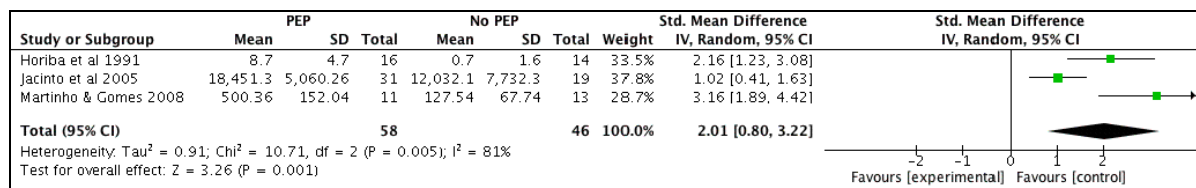
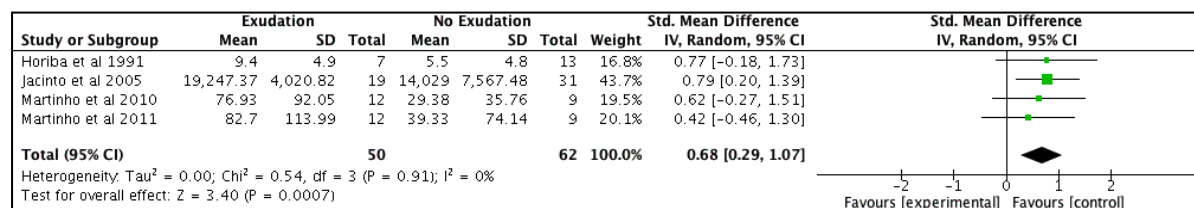


Figure 2. Forest plot with the evaluation of Exsudate (EX), previous episode of pain (PEP), pain on palpation (POP), size of radiographic lesion (SRL) and tenderness to percussion (TTP).

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis evaluating the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection. The data obtained in this study revealed that individuals having teeth with tenderness to percussion as well as previous episode of pain showed higher levels of endotoxin than their counterparts. Additionally, larger size of radiolucent area and presence of root canal exudation were associated with higher levels of endotoxin.

With regard to the methods used to quantify endotoxins, two studies used Gel Clot LAL assay (19,20), two used Endpoint Chromogenic LAL assay (9,13), three used Kinetic Turbidimetric LAL assay (1,11,12) and one compared all these three tests (2). All these LAL tests use the principle of a serine protease catalytic coagulation cascade activated by Factor C, the first component in the cascade which is a protease zymogen activated by endotoxin binding (37). This pathway activates downstream a proclotting enzyme into a clotting enzyme (i.e. coagulogen into coagulin) (37). The Gel Clot LAL assay and Kinetic Turbidimetric LAL assay use coagulogen by monitoring its conversion into coagulin, which begins to form a gel clot, thus increasing the turbidity. The Endpoint Chromogenic LAL assays uses synthetic peptide-pNA substrate, which is cleaved by the clotting enzyme, imparting a yellow color to the solution. The strength of the yellow color (determined at an optical density [OD] = 405 nm) resulting from chromogenic LAL substrate and turbidity (determined at an OD = 340 nm) due to the conversion of coagulogen is correlated with the endotoxin concentration from the standard curve. It is important to point out that the sensitivity of these tests depends on the time point (single or multiple time point) of the progress of the LAL reaction leading to the coagulogen conversion recorded. In the Endpoint Chromogenic LAL assay, OD is recorded at single time (at ≈16 minutes), which compromises its sensitivity (0.1-1 EU/mL). Conversely, in Kinetic Turbidimetric LAL assay, OD is read at multiple time points because the reaction proceeds with no termination step (z 60 minutes), which allows the concentration of endotoxin to be quantified over a wider range sensitivity (0.01-100 EU/mL in the turbidimetric methods).

The data obtained in this study revealed that individuals with tenderness to percussion showed higher contents of endotoxins than their

counterparts. According to the AAE glossary (38), percussion is a diagnostic procedure used to assess the condition of a body part by means of tapping, with a painful response indicating periradicular inflammation. The tooth, which is sensitive to percussion, has a periapical diagnosis of symptomatic apical periodontitis (39,40). Also, a correlation was found between higher levels of endotoxins and patients with previous episode of pain. These correlations are consistent with the hypothesis that LPS in clinical infections is related to the production of pain and mechanical allodynia. This hypothesis is strengthened by preclinical studies demonstrating that injection of LPS into rats produces nocifensive behavior and mechanical allodynia (41).

There are different mechanisms that can evoke pain due to bacterial LPS (25-27). It is well accepted that the innate immune response against bacterial contents leads to the release of different inflammatory mediators, including interleukin 1- beta ($IL-1\beta$), prostaglandin E2 (PGE2) and tumor necrosis factor alpha (TNF- α), which can sensitize nociceptors. Higher levels of PGE2 have been related to teeth with clinical symptomatology (3,42,43). Martinho et al. (3) found higher levels of PGE2 in macrophage supernatants stimulated with bacterial contents from teeth showing tenderness to percussion. Thus, higher contents of $IL-1\beta$ have also been detected in teeth with clinical symptomatology (44,45). Martinho et al. (1) not only indicated higher levels of endotoxins in teeth with tenderness to percussion, but also found higher levels of $IL-1\beta$ in macrophage supernatants stimulated with bacterial contents from teeth with this clinical symptomatology. Thereby, IL-1 is a potent stimulus for PGE-2 release (46).

Besides, increased sensitization and up-regulation of TRPV1 constitutes a potential mechanism by which TNF-alpha mediates inflammatory hyperalgesia and pain. It has been demonstrated that trigeminal neurons express both LPS receptors (TLR4 and CD14) leading to the hypothesis that bacterial byproducts might directly activate or sensitize trigeminal nociceptors. The sensitization and up-regulation of transient receptor potential cation channel, sub-family V, member 1 (TRPV1), has been addressed (26,27). Ferraz et al. (25), by using cultures of rat trigeminal neurons, demonstrated that pre-treatment with LPS produced a significant increase in the capsaicin-evoked release of calcitonin gene-related peptide (CGRP) compared to vehicle pre-treatment, thus showing sensitization of the capsaicin receptor TRPV1

by LPS. Additionally, the authors showed co-localization of the LPS receptor (toll-like receptor 4, TLR4) with CGRP-containing nerve fibers. Diogenes et al. (26) found that (i) LPS binds to receptors in trigeminal neurons by means of competitive binding, (ii) LPS evokes a concentration-dependent increase in the intracellular calcium accumulation (Ca^{2+})(i) and inward currents, and (iii) LPS significantly sensitizes TRPV1 to capsaicin measured by (Ca^{2+})(i) release of calcitonin gene-related peptide and inward currents. All these together imply that LPS is capable of directly activating trigeminal neurons and sensitizing TRPV1 via a TLR4-mediated mechanism.

Our results revealed that higher levels of endotoxins were associated with the presence of exudation in infected root canals. Exudate is defined as fluid, cells and plasma proteins which escaped from the vascular system and accumulates in a tissue or tissues, usually being the result of inflammation (38). The presence of exudation in root canal reflects an acute inflammation in periapical lesion (42). Gram-negative bacterial LPSs are one of the mainly potent stimuli for macrophage cells in the release of PGE2 (47,48). Martinho et al. (3) revealed a positive correlation between number of Gram-negative bacterial species and levels of PGE2 macrophage secretion. PGE2 is both directly and indirectly implicated in most of the inflammatory and destructive changes occurring in apical lesions (e.g., vasodilatation), thus increasing vascular permeability and collagen degradation (49). Takayama et al. (42) stated that the PGE2 concentration in periapical exudate could reflect the state of the disease activity in periapical periodontitis. Additionally, IL-1 β and TNF- α are also implicated in exudation (50-53). Therefore, a more complex network among these and other different cytokines seems to play a role in exudation (32).

Higher levels of endotoxin have been found in root canals with larger size of radiolucent (>2mm). Previous studies demonstrated that the endotoxin content of teeth with radiolucent areas is five times as great as that of teeth without them (54,55). Such a correlation is not inconsistent with the hypothesis that LPS in clinical infections is related to bone destruction. This hypothesis is strengthened by pre-clinical studies showing that injection of LPS into animal tissues induces periapical bone destruction (30,56,57). The LPS released from the infected root canal triggers the synthesis of IL-1 and TNF- α from macrophages, which in turn up-regulates the

production of MMP-1 (30) and serves primarily for degrading non-mineralized extracellular matrix (58,59). Stimulation of osteoclastogenesis by MMP-1 and generating collagen degradation fragments on bone surfaces has also been proposed (60). Studies have also demonstrated that MMP-1-expressing macrophages increase consistently as the lesion expands, which implies the involvement of MMP-1 in the production of macrophages in periapical destruction (61,62). These authors have also shown that LPS induces the expression of inducible nitric oxide synthesis (iNOS) and transforming growth factor- β 1 (TGF- β 1) genes (63,64). Furthermore, LPS stimulates bone resorption by enhancing the receptor activator of NF- κ B ligand (RANKL) through activation of toll-like receptor 2 in osteoblasts (31) as well as by inhibiting osteoblast differentiation (29). *Porphyromonas endodontalis* LPS inducing RANKL by osteoblast (65) and *Porphyromonas gingivalis* exacerbating ligature-induced RANKL-dependent alveolar bone resorption via differential regulation of toll-like receptor 2 (TLR2) and TLR4 (66) are reported. Martinho et al. (32) have demonstrated that the complex interplay between different cytokines plays a role in periapical bone destruction, including significant (-) correlations between IL-6 and IL-1 β as well as PGE2 and (+) correlation between TNF- α and PGE2.

It is important to highlight that not only the levels of endotoxin are implicated with the presence/development of symptoms and severity of bone destruction, but also the bacterial community involved in the infection, its interplay (synergism/antagonism) (2,6,67), and consequently, the type of bacterial LPS and its lipid A structure (68,69). Among the Gram-negative bacteria, black-pigmented anaerobes, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* have been implicated as pathogens associated with the presence/development of symptoms and severity of bone destruction (2,6,7,67). Importantly, the LPS structure shows considerable heterogeneity among bacterial species (69-71), thus evoking different patterns of inflammatory response (33,50,69). It is worth to point out that the lipid A structure of the LPS molecule (i.e. hydrophobic component located in the outer leaflet of the outer membrane, responsible for the toxic effects of infections with Gram-negative bacteria) (72) can suffer modulation from the environment, particularly, regarding hemin concentration (70) and temperature (73). Hence, LPS molecule can also vary among different strains of

single species, and consequently exhibit different inflammatory potentials (70,71,73).

The studies investigated in this systematic review are the best evidence available. Nevertheless, they present various limitations in terms of study design, heterogeneity, controls, sample sizes, and other methodological aspects. Despite these limitations, our study provides strong evidence that endotoxin play an important role in endodontic infections.

Overall, this meta-analysis provides strong evidence that endotoxin are related with the presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

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2.2 Artigo – Rabello DGD, Corazza BJ, Gomes APM, Ferreira LL, Santamaria MP, Martinho FC. A terapia fotodinâmica suplementar otimiza a desinfecção de bactérias e endotoxinas em uma e duas visitas no tratamento endodôntico? Um ensaio clínico randomizado. *Does supplemental photodynamic therapy optimize the disinfection of bacteria and endotoxins in one-visit and two-visit root canal therapy? A randomized clinical trial* *

RESUMO

Sabendo-se que o preparo químico-mecânico (PQM) não é capaz de eliminar por completo microrganismos e seus subprodutos presentes nas infecções endodônticas, foi proposta um ensaio clínico randomizado utilizando a Terapia Fotodinâmica (PDT) como tratamento suplementar ao PQM. Com isto o estudo avaliou e comparou a efetividade da suplementação da terapia endodôntica com o uso de PDT na redução microbiana, bem como na redução de endotoxinas presente nas infecções endodônticas primárias nas modalidades de terapia endodôntica em única e múltiplas sessões. Para isto selecionou-se 24 casos de dentes com infecção endodôntica primária que foram aleatoriamente divididos em 2 grupos (n=12): SU - sessão única e SM – sessão múltipla. Foram realizadas coletas microbiológica e de endotoxinas dos canais radiculares utilizando cone de papel estéril/ apirogênico em diferentes momentos operatórios. Foi realizado cultura microbiana para determinar a carga bacteriana em UFC/mL (Unidades Formadoras de Colônias/mL) e para quantificação de endotoxinas foi realizado o teste KQCL de “Lisado de Amebócito Limulus” (LAL). Para carga microbiana, a suplementação do tratamento em sessão única com PDT reduziu significativamente os níveis bacterianos ($p < 0,05$), mas não nos casos de duas sessões ($p > 0,05$). Para os níveis de endotoxinas, na sessão única e de duas sessões, não houve diferença significativa após uso de PDT ($p > 0,05$).

Palavras-chave: Periodontite periapical. Tratamento do canal radicular. Desinfecção. Terapia fotodinâmica. PDT. Endotoxina.

ABSTRACT

To evaluate the effectiveness of supplemental photodynamic therapy (PDT) in optimizing the removal of bacteria and endotoxins from primarily infected root canals after one-visit and two-visit treatments. 24 primarily infected root canals with apical periodontitis were selected and randomly divided into one-visit (n=12) and two-visit (n=12). Samples were collected before and after root canal procedures. Endotoxins were quantified by chromogenic limulus amebocyte lysate assay. Culture techniques were used to determine bacterial colony-forming unit counts. Bacteria and endotoxins were detected in 100% of the initial samples, with median values of 1.97×10^5 CFU/mL and 24.983 EU/mL, respectively. The CMP using single-file reciprocating technique was effective in the reduction of bacteria and endotoxins (All, $p < .05$). The supplemental PDT was effective in reducing bacterial load in the one-visit ($p < .05$) but not in the two-visit treatment after use of Ca(OH)_2 medication for 14 days ($p > .05$). In the two-visit group, after 14 days of inter- appointment medication with Ca(OH)_2 , a

*Artigo elaborado de acordo com as normas do Periódico *International Endodontic Journal*. Submetido em: 08/08/2016.

CMP alone (from 1.041 to 0.094 EU/mL)($p < .05$). Despite the type of treatment, the supplemental PDT was not effective against endotoxins ($p > .05$). The photodynamic therapy optimized the disinfection of bacteria from root canals in one-visit but not for two visit treatment modality with the accomplishment of calcium hydroxide medication. Thus, PDT is not effective against endotoxins.

Keywords: Bacterial reduction. Disinfection. Endodontic treatment. PDT. Photodynamic therapy. Endotoxin.

INTRODUCTION

It has long been known that an effective disinfection of the root canal systems has not only to do with elimination of bacteria, removal of dead cells and dissolving of pulp tissue (Clarkson et al. 2012, Guneser et al. 2015), but also with debridement and eradication of biofilms (Case et al. 2012) as well as elimination and neutralization of bacterial virulence factors (Martinho & Gomes, 2008, Martinho et al. 2010, Xavier et al. 2013).

The knowledge of the microbial ecology involved in the infection is a key step for determining an optimal disinfection protocol (Tzanetakis et al. 2015). It is known that primary endodontic infection is polymicrobial, comprising both Gram-positive and Gram-negative bacterial species (Martinho et al. 2014, Tzanetakis et al. 2015). Lipopolysaccharide (LPS, also known as endotoxin) is the main virulence factor found in the outer membrane of the bacterial cell (Fabricius et al. 1982), exhibiting high biological activity (Hong et al. 2004). Endotoxin has been detected in 100% of the primarily infected teeth (Martinho et al. 2014, Cardoso et al. 2015), with higher levels of LPS being correlated with development of symptoms and severity of bone destruction (Schein & Schilder, 1975, Horiba et al. 1991, Martinho & Gomes 2008, Martinho et al. 2011, Sousa et al. 2014, Cardoso et al. 2015).

Despite the advances in instrumentation techniques with the use of reciprocating single-instrument (Martinho et al. 2010, Martinho et al. 2014, Neves et al. 2016) or multiple NiTi files systems in continuous rotation motion (Cardoso et al. 2015, Neves et al. 2016), studies preconizing one- and two-visit treatment approach have failed in demonstrating an optimal disinfection of the

root canal system (Martinho et al. 2010, Xavier et al. 2013, Martinho et al. 2014, Vera et al. 2012, Ferreira et al. 2015).

In the search of supplemental methods to optimize the root canal disinfection, photodynamic therapy (PDT) has been considered in the past years (Bonsor et al. 2006, Garcez et al. 2008, Garcez et al. 2010, Fimple et al. 2008, Soukos et al. 2006, Cherpa et al. 2014). However, few clinical studies evaluated the effectiveness of PDT in the disinfection of the root canal systems (Bonsor et al. 2006, Garcez et al. 2008, Garcez et al. 2010). Moreover, up to now, there is no clinical study evaluating the effectiveness of PDT in reducing and/or eliminating endotoxins from primarily infected root canals. To address, this study evaluated the effectiveness of supplemental photodynamic therapy (PDT) in optimizing the removal of bacteria and endotoxins from primarily infected root canals after one-visit and two-visit treatments.

MATERIAL AND METHODS

This study was designed as a randomized clinical trial, which was registered and approved by the institutional Review Board (protocol # NCT02824601). The local human research ethics committee approved the protocol describing the sample collection for this investigation, with all the patients being volunteers and signing an informed consent form. Sample size calculation: the minimum sample size was determined to be 12 teeth per group on the basis of a 10% mean difference in outcome between the groups and power = 0.80 ($P < .05$). Randomization, allocation, concealment and blinding: An external person generated a random allocation sequence in a computer program. This sequence was placed in opaque, sealed envelopes. Each envelope included the randomization code for each patient, which was only revealed during the treatment procedure, just after the chemo-mechanical preparation. It was ensured that both the patient and the operator were unaware of the treatment protocol assigned until completion of chemo-mechanical preparation. After, the blinding of the examiners was not practical because the application of PDT after chemo-mechanical preparation (CMP) in the 1-visit group or the placement of Ca(OH)₂ medication in the 2-visit group.



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	1
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	1-2
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	
	4b	Settings and locations where the data were collected	3
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5-6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5-6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	3
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	3
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	3
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	3
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	3
		assessing outcomes) and how	
Statistical methods	11b	If relevant, description of the similarity of interventions	N/A
	12a	Statistical methods used to compare groups for primary and secondary outcomes	7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8-9
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	8-9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	N/A
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	N/A
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	11-15
Other information			
Registration	23	Registration number and name of trial registry	3
Protocol	24	Where the full trial protocol can be accessed, if available	Clinicaltrials.gov
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming for those and for up to date references relevant to this checklist, see www.consort-statement.org

Patient Selection

Twenty-four patients attending the Dental School of the São Paulo State University (UNESP), São José dos Campos, Brazil, for primary endodontic treatment were included in the present study. The demographic data from the patients that participated in this study is shown in table 1. A detailed dental and general health history was obtained from each patient. Those who had received antibiotic treatment during the past three months were excluded. All the teeth were single-rooted with primary endodontic infection and showing presence of one root canal and absence of periodontal pockets deeper than 4 mm as well as root canal calcification. Teeth were instrumented according to the manufacturer instructions. When the anatomy allowed the passive entrance of K-file #30 to the working length (WL), it was used the R50 Reciproc file. If K-file #20 was the one that fitted in the canal, than it was used the R40 Reciproc file. When the K-file #20 was not able to get to the WL passively, than Reciproc R25 was used instead. The teeth that could not be isolated with rubber dam were also excluded.

Sampling/Treatment Procedures

Files, instruments, and all the materials used in this study were treated with Co60 gamma radiation (20 kGy for 6 hours) for sterilization and elimination of pre-existing endotoxins (EMBRARAD; Empresa Brasileira de Radiação, Cotia, SP, Brazil). The method used for disinfection of the operative field had been previously described elsewhere (Martinho et al. 2014). Briefly, the teeth were isolated with rubber dam. The crown and surrounding structures were disinfected with 30% H₂O₂ (volume/volume [V/V]) for 30 seconds, followed by 2.5% NaOCl for the same period of time and then inactivated with 5% sodium thiosulfate. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it onto blood agar plates, which were then incubated both aerobically and anaerobically.

A two-stage access cavity preparation was made without the use of water spray, but under manual irrigation with sterile/apyrogenic saline solution and by using sterile/apyrogenic high-speed diamond bur. The first stage was performed for a major removal of contaminants, including carious lesion and restoration. In the second stage, before entering the pulp chamber, the access cavity was

disinfected according to the protocol described above. Sterility of the internal surface of the access cavity was checked as previously described and all procedures were performed aseptically. For the one-visit group, root canal samplings were performed at the baseline, immediately after chemo-mechanical preparation, and after PDT; for the two-visit group, root canal samplings were performed at the baseline, immediately after chemo-mechanical preparation, after 14-days of intracanal medication, and after PDT.

For endotoxin samples, a sterile/apyrogenic paper points (size #15, Dentsply-Maillefer, Balaigues, Switzerland) was introduced into the full length of the canal, which was determined radiographically, and retained in position during 60 seconds for sampling (Martinho et al. 2014). Immediately after, the sample was placed in pyrogen-free glass and immediately suspended in 1 mL of limulus amebocyte lysate (LAL) water (Lonza, Walkersville, MD, USA) and frozen at -80 °C for further study of endotoxins by using kinetic chromogenic limulus amebocyte lysate (LAL) assay (Lonza, Walkersville, MD, USA). For bacterial samples, this sampling procedure was repeated with 3 paper points, which were then pooled in a sterile tube containing 1mL of viability medium Göteborg agar III (VMGA III).

Next, the root canal length was determined with preoperative radiograph and confirmed by using an apex locator (RomiApex A-15; Romidan Dental Solution, Kiryat-Ono, Israel). The root canals were prepared by using the appropriate Reciproc file (VDW, München, Germany), which was used in reciprocating motion powered by a torque-limited electric motor (VDW Silver, VDW) with preset adjustments. The instrument was used in an in-and-out pecking motion of about 3 mm in amplitude with apical pressure. After three pecking motions, the instrument was removed from the canal, which was irrigated with 2.5% NaOCl (Martinho et al. 2014). Next, a #15 K-type file (Dentsply, Maillefer, Balaigues, Switzerland) was inserted at the working length (WL) to check whether the canal was patent. These procedures were repeated until the Reciproc instrument reached the WL. Irrigation throughout the procedures was performed by using disposable syringe and 30-G NaviTip needle (Ultradent, South Jordan, UT, USA). Each root canal was instrumented with a single Reciproc instrument, and each instrument was used to prepare only one canal. Before the second sampling after CMP, the root canals were irrigated with 17% EDTA

for 3 minutes, followed by 5 mL of 2.5% NaOCl solution, which was inactivated with 5 mL of sterile 0.5% sodium thiosulfate during 1 minute before being removed with 5 mL of LAL water.

After CMP, In the one-visit group, the root canal was washed thoroughly with sterile/apyrogenic saline solution and dried with sterile/apyrogenic paper points (Dentsply, Maillefer, Balaigues, Switzerland). Next, the photosensitizer methyl blue (São Carlos, São Paulo, Brazil) was placed into the root canal (0.5 mL) by using an endodontic micro needle until a complete fill has been achieved and then agitate for 60 s inside the root canal by using a #25 K-type file (Dentsply, Maillefer) to ensure that methyl blue reached the full extension of the root canal. The illumination was performed with a 300- μ m diameter fiber coupled with a diode laser (MMOptics, São Carlos, SP, Brazil). The fiber was placed in the apical portion of the root canal at a point where resistance to the fiber could be felt with spiral movements (from apically to cervically) manually performed to ensure full diffusion of light inside the canal lumen (Garcez et al. 2008, 2010). These movements were repeated 10 times per minute (Garcez et al. 2008, 2010). The laser emitted a 660-nm light at a total power of 60 mW for 120 seconds. Next, a new bacteria and endotoxin samples were performed as described previously.

Particularly, in the two-visit group, after CMP, the root canal was filled with freshly prepared paste of $\text{Ca}(\text{OH})_2$ in saline solution for a period of 14 days. $\text{Ca}(\text{OH})_2$ paste was inserted into the root canals with the aid of a lentulo spiral filler (Dentsply, Maillefer). Care was taken to properly fill the root canal with $\text{Ca}(\text{OH})_2$ paste. The paste was plugged at the canal orifice level by using a sterile cotton pellet. Next, the access cavity was properly closed with ionomer cement. After 14 days of intracanal medication, the tooth was isolated and disinfected and the provisional restoration was removed. Next, the root canals were irrigated with 10 mL of LAL water, and calcium hydroxide medication was neutralized with 0.5% citric acid (Sigma-Aldrich, St. Louis, Missouri, USA). Afterwards, the root canals were irrigated with 10 mL of LAL water and dried with sterile/ apyrogenic paper points, and PDT was performed as previously described.

Bacterial Procedures

Quantification of Total Culturable Bacterial Counts

In the present study, the method used for culture procedures was previously reported by the author elsewhere (Martinho et al. 2014). Briefly, the transport media containing the root canal samples were thoroughly shaken for 60 seconds (Vortex; Marconi, Piracicaba, São Paulo, Brazil). Serial 10-fold dilutions were made up to 10-in tubes containing fastidious anaerobe broth (FAB; Lab M, Bury, UK). Fifty microliters of the serial dilutions was plated onto 5% defibrinated sheep blood fastidious anaerobe agar (FAA; LabM) by using sterile plastic spreaders to culture non-selectively obligate anaerobes and facultative anaerobes. The plates were incubated at 37°C in anaerobic atmosphere for up to 14 days. After this period, colony-forming units (CFUs) were visually quantified for each plate.

Endotoxin Procedures

Quantification of Endotoxin Concentration

The kinetic chromogenic LAL assay (Lonza; São Paulo, São Paulo, Brazil) was used for quantification of endotoxins, with Escherichia coli endotoxin being used as standard. A positive control (root canal sample contaminated with a known amount of endotoxin) was included for each sample to determine the presence or absence of interfering agents. For the test, 100 μ L of apyrogenic water (reaction blank), five standard endotoxin solutions (0.005–50 endotoxin units in EU/mL), root canal samples, and positive controls (root canal samples contaminated with a known concentration of endotoxin, i.e. 10 EU/mL) were added to a 96-well apyrogenic plate. The tests were carried out in quadruplicate. The plate was incubated at 37°C $\pm$ 1°C for 10 minutes in a Kinetic-QCL (Lonza) reader, which was coupled to a microcomputer by means of the WinKQCL software. Next, 100 μ L of chromogenic reagent was added to each well. After the beginning of the kinetic test, the software continuously monitored absorbance at 405 nm in each microplate well and automatically calculated the log/log linear correlation between reaction time of each standard solution and corresponding endotoxin concentration.

Statistical Analysis

The data collected (CFUs and endotoxin concentrations) were statistically analysed by using the SPSS for Windows (SPSS Inc., Inc, Chicago, IL, USA). The sample size calculation revealed that 12 teeth per group would suffice to show a 5% difference in bacterial counts with a power of 80%. Repeated measure ANOVA and Tukey's tests were used to for intragroup analysis. One-Way ANOVA and Tukey's test was used for intergroup analysis. The significance level was always set at 5% ($P < .05$).

RESULTS

A total of 24 patients, being 15 males and 9 females, participated in this study. Random allocation of subjects resulted in 12 patients in the 1-visit-treatment group (7 males and 5 females) and 12 patients in the 2-visit-treatment group (8 males and 4 females) (Table 1). The mean age of patients in 1-visit-treatment group was 48 ± 15.49 whilst it was 38.6 ± 13.97 in the two-visit-treatment group (Table 1).

Recruitment was performed from February/ 2015 until June/ 2016. The trial was completed in June/2016. Figure 1 shows the participant flow chart.

Determination of Total Cultivable Bacterial Counts

Bacteria were found in all baseline samples of the 24 primarily infected root canals investigated, with total bacterial count ranging from 1.09×10^4 to 5.38×10^6 CFU/mL (median value, 1.97×10^5 CFU/mL). Individual, median and range values of bacterial counts found in the one-visit and two-visit groups for the baseline samples are shown in Table 2. The chemo-mechanical preparation using single-file reciprocating instrumentation and irrigation with 2.5% NaOCl and 17% EDTA substantially reduced the median value of total bacterial load in both one-visit (from 1.94×10^5 to 7.01×10^2 CFU/mL) and two-visit groups (from 2.01×10^5 to 6.73×10^2 CFU/mL) (all, $p < .05$) (Table 2). Intergroup comparison of total CFU counts found after instrumentation showed no difference between one-visit and two-visit groups ($p > .05$). The median percentage values of bacterial reductions were 95.18% and 96.07% for one-visit and two-visit treatments, respectively (Table 2). After CMP, bacteria were still recovered from infected root

canals in both one-visit (8/15) and two-visit (6/15) groups. In the one-visit group, the supplemental PDT not only significantly decreased the level of bacteria (from 7.01×10^2 to 1.16×10^2 CFU/mL) ($p < .05$), but also decreased the number of root canals yielding positive culture after CMP alone (8/15 after CMP; 5/15 after PDT). The median percentage values of bacterial reductions increased from 95.18% to 98.70%. After 14 days of inter-appointment medication with Ca(OH)_2 , a significant median reduction of bacterial load was found in the two-visit treatment after CMP alone (from 6.73×10^2 to 1.22×10^2 CFU/mL) ($p < .05$) (Table 2). Thus, an increase in the median percentage value of bacterial reduction was observed (from 96.07% to 99.05%). The supplemental PDT in the two-visit group showed no significant improvement in the reduction of bacterial load after use of Ca(OH)_2 medication for 14 days (reduction from 1.19×10^2 compared to 1.22×10^2 CFU/mL) ($p > .05$) (Table 2).

Quantification of Endotoxin Concentration

The standard curve for detection of endotoxins fulfilled the criteria of linearity ($r=1$). With regard to the baseline samples, the LAL-assay indicated the presence of endotoxins in 100% of the root canal samples (24/24), with a median value of 24.983 EU/mL (ranging from 7.490 to 47.710 EU/mL). Individual median and range values of bacterial counts found in the one-visit and two-visit groups for the baseline samples are shown in Table 3. With regard to the CMP, the use of reciprocating single-instrument and irrigation with 2.5% NaOCl and 17% EDTA reduced the median levels of endotoxin in both one-visit (from 24.942 to 1.920 EU/mL) and two-visit groups (from 25.017 to 1.041 EU/mL) groups (all $p < .05$) (Table 3). The median percentage values of endotoxin reduction were 94.91% and 95.03% for one-visit and two-visit treatments, respectively (Table 3). After CMP, endotoxin was still recovered from 100% of the root canal samples (Table 3). Particularly, in the one-visit group, the supplemental PDT did not optimize the removal/elimination of endotoxins achieved with CMP alone as shown in table 3, and endotoxins were still detected in 100% (12/12) of the root canal samples. In the two-visit group, after 14 days of inter- appointment medication with Ca(OH)_2 , a significant reduction in the median levels of endotoxins was found in comparison

to CMP alone (from 1.041 to 0.094 EU/mL) ($p < .05$) (Table 3), with only 4/12 root canals being positive for endotoxins.

Particularlry, the supplemental PDT in the two-visit group showed no significant improvement in the reduction of endotoxin levels after the use of Ca(OH)₂ medication for 14 days (i.e., reduction from 0.094 to 0.081) ($p > .05$). Thus, the intergroup analysis revealed that the two-visit treatment was more effective in the removal of endotoxins than one-visit treatment ($p < .05$).

Table 1. Demographic data of the study.

Group	Male/female	Age (years)
1-visit-treatment	7/5	48 ± 15.49
2-visit-treatment	8/4	38.6 ± 13.97
Total	15/9	40.3 ± 14.36

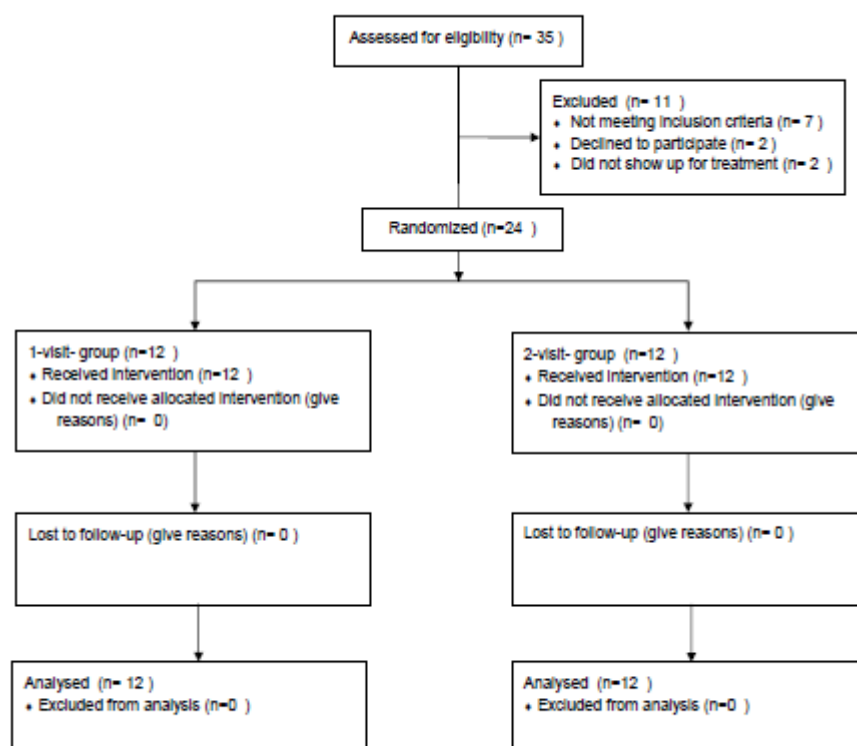


Figure 1. Consolidated standards of reporting trials flow chart.

Table 2. Distribution of the median/ range values of bacteria load (CFU/mL) found in root canals at different sampling times, median percentage values of bacterial load reduction and number of root canals with positive culture.

Sampling times	1-Visit Treatment		
	Bacterial Load (CFU/mL) Median/ Range values	Median % of reduction	# of root canals with positive cultures
Before chemomechanical preparation	1.94×10^5 (1.09×10^4 - 4.39×10^5) Aa	x	12/12
After chemomechanical preparation	7.01×10^2 (0 - 3.45×10^3) Bb	95.18%	6/12
After supplementary-PDT therapy	1.16×10^2 (0 - 1.35×10^2) Cc	98.70%	3/12
Sampling times	2-Visit Treatment		
	Bacterial Load (CFU/mL) Median/ Range values	Median % of reduction	# of root canals with positive cultures
Before chemomechanical preparation	2.01×10^5 (1.27×10^4 - 5.38×10^5) Aa	x	12/12
After chemomechanical preparation	6.73×10^2 (0 - 2.94×10^3) Bb	96.07%	7/12
After Ca(OH) ₂ interappointment medication	1.22×10^2 (0 - 1.93×10^2) Cc	99.05%	3/12
After supplementary-PDT therapy	1.19×10^2 (0 - 1.47×10^2) Cc	99.13%	2/12

- Intra-group analysis – different capital letters = $p < .05$.

- Inter-group analysis - different lower case letters = $p < .05$.

Table 3. Distribution of the median/ range values of endotoxins (EU/mL) found in root canals at different sampling times, median percentage values of endotoxins reduction and number of root canals positive for endotoxins.

Sampling times	Endotoxin contents (EU/mL) Median/ Range values	Median % of reduction	# of root canals positive for endotoxins
Before chemo-mechanical preparation	24.941 (8.293-67.100) Aa	x	12/12
After chemo-mechanical preparation	1.920 (0.054-4.310) Bb	95.03%	12/12
After supplementary-PDT therapy	0.718 (0.184-3.479)Bb	96.11%	12/12
2-Visit Treatment			
Sampling times	Endotoxin contents (EU/mL) Median/ Range values	Median % of reduction	# of root canals positive for endotoxins
Before chemo-mechanical preparation	25.067 (7.490 - 43.095) Aa	x	12/12
After chemo-mechanical preparation	1.041(0.083-3.910) Bb	94.91%	12/12
After Ca(OH)2 medication	0.094 (0.03-0.147)Cc	98.01%	4/12
After supplementary-PDT therapy	0.081 (0.029-0.480) Cc	98.16%	4/12

- Intra-group analysis – different capital letters = $p < .05$.

- Inter-group analysis - different lower case letters = $p < .05$.

DISCUSSION

In the present study, although a significant bacterial load reduction was achieved by using single-file reciprocating instrumentation and irrigation with 2.5% NaOCl and 17% EDTA (from 10^5 to 10^2 UFC/mL), culturable bacteria were still recovered from 50% of the infected root canals treated in the one-visit group. The limited ability of single-visit treatment on eliminating bacteria from root canal infection has been demonstrated by previous studies in which culturable bacteria could still be recovered in $\approx$ 30-50% (Xavier et al. 2013, Martinho et al. 2014, Neves et al. 2016). It is important to highlight that higher success rates of endodontic therapy are reported in teeth with negative bacterial culture (Molander et al. 2007, Sjögren et al. 1997). Thus, the persistence of culturable bacteria at the time of obturation has shown to reduce such rate (Sjögren et al. 1997, Molander et al. 2007).

Considering the limitation of CMP in eliminating bacteria and the search for a supplemental strategy to optimize the root canal disinfection, our clinical study has addressed the supplemental PDT for one-visit and two-visit treatments. PDT is based on the use of photosensitizer activated with low-energy light for production of free-radical species (e.g. singlet oxygen) (Hamblin & Hasan 2004), which is highly reactive and is known to target different bacterial sites, including cell wall, nucleic acid and membrane proteins (George & Kishen, 2008). All these confer advantages such as broad-spectrum antibacterial activity and low risk of resistance.

The effectiveness of PDT depends on different aspects, including photosensitizer and its time of application, light wavelength (nm), laser type and power (mW), emission laser time (Cherpa et al. 2014), type of bacterial infection (whether Gram-positive or Gram-negative), and bacterial growth mode (Upadya & Kishen, 2010).

*With regard to the photosensitizer itself, phenothiazines and xanthenes are the two classes commonly tested for antibacterial efficacy (Soukos et al. 2006, Shrestha & Kishen 2012). We have selected the methylene blue (MB), which falls in the category of cationic phenothiazines and is effective against different endodontic bacteria such as *Enterococcus* ssp. and drug-resistant*

strains (Wainwright 1990, Soukos et al. 2006, George & Kishen 2008). The high antibacterial effect of MB is attributed to its hydrophilicity, low molecular weight, cationicity (George & Kishen 2008) and high production of singlet oxygen (Shrestha & Kishen 2012). Shrestha & Kishen (2012) reported that the singlet oxygen production is less impaired by pulp, dentin, and LPSs present in root canals when using MB than other photosensitizer.

Particularities of the mechanism of bacterial inactivation by MB are described in the literature (Wainwright et al. 1999), which is extensive regarding for phenothiazine dyes (Wainwright et al. 1999, Tardivo et al. 2005). Of clinical relevance, our data have demonstrated that the one-visit treatment using single-file reciprocating instrumentation, irrigation (2.5% NaOCl and 17% EDTA) and PDT (application of methyl blue photosensitizer for 60 seconds exposed to diode laser at 660 nm wavelength for 60 seconds and potency of 60 mW for 120 seconds) significantly decreased the levels of bacteria from infected root canals (from 7.01 to 1.16×10^2 CFU/mL). Bonsor et al. (2006) using diode laser in similar In the two-visit group, the use of $\text{Ca}(\text{OH})_2$ medication for 14 days significantly improved the reduction of bacterial load and also yielded a higher number of root canals free of culturable bacteria (3/12) compared to CMP alone by itself (7/12), thus corroborating previous findings (Siqueira et al. 2007, Ferreira et al. 2015). Particularly, in the two-visit group, the supplemental PDT showed no significant improvement in the median percentage values of bacterial reduction achieved with 14-day application of $\text{Ca}(\text{OH})_2$ medication. In contrast, Garcez et al. (2008) performing a weekly treatment using $\text{Ca}(\text{OH})_2$ medication and a two session of chemo-mechanical debridement followed by PDT application indicated a significant bacterial load reduction (99.90%) compared to the first session of treatment after hand instrumentation up to # 40.02 apical file enlargement. Although our data revealed no significant improvement in bacterial disinfection with supplemental PDT after 14-days of $\text{Ca}(\text{OH})_2$ medication, a 99.05% of bacterial reduction was achieved with 14-days of $\text{Ca}(\text{OH})_2$ medication, which is close to 99.90% of disinfection indicated by Garcez et al. (2008). It is important to highlight that there was no significant difference in the bacterial load reduction found after 14 days of $\text{Ca}(\text{OH})_2$ medication and after one-visit treatment with supplemental PDT.

To the best of our knowledge, this is the first clinical study evaluating the ability of supplemental PDT to improve the removal of endotoxins from primarily infected root canals after CMP in one-visit and two-visit groups. Gram-negative bacterial endotoxins (LPSs), which exhibit a high biological toxicity and are related to the development of symptoms and severity of bone destruction, were detected in 100% of the primarily infected teeth in our study agreeing with previous investigations (Martinho et al. 2010, 2013, 2014). Our results have revealed that CMP by using single-file reciprocating instrumentation and irrigation with 2.5% NaOCl a 17% EDTA decreased significantly the levels of endotoxin in both one-visit (from 24.942 to 1.920 EU/mL) and two-visit (from 25.017 to 1.041 EU/mL) groups. Despite such a reduction, the present study has shown the limited ability of CMPs in eliminating endotoxins from primarily infected teeth as endotoxins were recovered from 100% of the root canals after instrumentation. Such finding is in agreement to previous investigations (Martinho et al. 2010, 2013, 2014).

Additionally, our results revealed not only the ability of Ca(OH)_2 (when used as an inter-appointment medication for 14 days) to enhance the reduction of endotoxins levels found in root canal infection (from 1.041 to 0.094 EU/mL) but also to eliminate endotoxins from the majority of the root canals (8/12). The ability of Ca(OH)_2 to neutralize endotoxins in root canals when used as intracanal medication demonstrated in this study is in accordance with previous studies (Xavier et al. 2013, Ferreira et al. 2015). The ability of Ca(OH)_2 in neutralizing LPS molecule is attributed to its capacity to cleavage ester-linked hydroxy fatty acids (Safavi & Nichols, 1993).

Regardless of the type of treatment (i.e., one-visit or two-visit treatment), the supplemental PDT had no significant effectiveness in reducing the endotoxin levels or in increasing the number of endotoxin-free root canals. Shrestha et al. (2012) reported that LPSs decrease the antimicrobial efficacy of PDT. Thus, the higher antimicrobial activity against Gram-positive than Gram-negative bacteria is discussed. Although Gram-positive bacteria possess a porous layer of peptidoglycan and a single lipid layer, Gram-negative bacteria have a double lipid bilayer surrounding the peptidoglycan layer plus an outer layer of lipopolysaccharide, which limits the diffusion of photosensitizer agent into the

bacterial cytosol (Sharma et al. 2011) and consequently the antimicrobial activity of PDT.

Finally, the limitations of a clinical study must be taken into consideration. The levels of endotoxins found in root canal infection varied among different patients, and consequently, varied among the groups tested. Thus, although an attempt was made to fill all the canals completely with calcium hydroxide intracanal medication, the completeness of fill and the quantity of intracanal medication in the canals could not be verified.

CONCLUSION

In conclusion, the photodynamic therapy optimized the disinfection of bacteria from root canals in one-visit but not for two visit treatment modality with the accomplishment of calcium hydroxide medication. Thus, photodynamic therapy is not effective against endotoxins.

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3 CONSIDERAÇÕES GERAIS

Dentre os nossos estudos sobre infecção do canal radicular, foi realizado uma revisão sistemática com metanálise com o intuito de avaliar a relação entre níveis de endotoxinas e a presença de sinais e sintomas clínicos, bem como de sinais radiográficos em pacientes com infecção endodôntica.

Para isto uma busca eletrônica foi realizada por dois autores, no idioma inglês, nas bases de dados Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge e Scopus. Entre 285 artigos identificados, 29 foram incluídos para leitura completa e 8 artigos foram incluídos por estarem dentro dos critérios de inclusão.

Os dados obtidos neste estudo revelaram que indivíduos com dor à percussão apresentaram maior concentração de endotoxinas que aqueles que não apresentaram este sintoma. De acordo com glossário AAE, percussão é o método de diagnóstico usado para avaliar a condição de parte do corpo através de leves toques, cuja resposta à dor indica uma inflamação periradicular (American Association of Endodontists, 2015) O dente que é sensível à percussão, tem o diagnóstico de periodontite periapical assintomática (Zero *et al.*, 2011; Owatz *et al.*, 2007). Também correlação foi encontrada entre maiores níveis de endotoxinas e episódio prévio de dor. Estas correlações são consistentes com a hipótese que LPS em infecções clínicas é relacionado com a produção de dor e mecanismo de alodínia. Esta hipótese é fortalecida por estudos pré-clínicos demonstrando que injeções de LPS em ratos provocam comportamento nocifensivo e mecanismo de alodínia. (Cahill *et al.*, 1998)

Há diferentes mecanismos que podem provocar dor devido ao LPS bacteriano (Ferraz *et al.*, 2011; Diogenes *et al.*, 2011; Khan *et al.*, 2008). É bem aceito que resposta imune inata contra conteúdo bacteriano inicia a liberação de diferentes mediadores inflamatórios, incluindo interleucina 1- beta (IL-1 β), prostaglandinas E2 (PGE2) e fator de necrose tumoral alpha (TNF- α) que podem sensibilizar nociceptores. Maiores níveis de PGE2 tem sido associado com sintomatologia clínica (Martinho *et al.*, 2013; Takayama *et al.*, 1996; McNicholas *et al.*, 1991). Martinho e colaboradores em 2010, encontraram maiores níveis de PGE2

em macrófagos estimulados com sobrenadantes de conteúdo bacteriano com dentes mostrando dor à percussão. Então, maiores concentrações de IL-1 β tem sido também detectados em dentes com sintomatologia (Lim *et al.*, 1994; Kuo *et al.*, 1998). Portanto, IL-1 β é um potente estimulante para lançamento de PGE-2 (46).

A metanálise revelou que indivíduos com dor à percussão e episódio de dor prévio (PEP) apresentaram maior nível de endotoxina que os casos que não apresentaram nenhum destes sintomas. O tamanho da área radiolúcida (SRL) > 2 mm e presença de exudação (EX) estavam associados com maior nível de endotoxinas. Esta metanálise mostra evidência de que endotoxina está relacionado com presença de sintomas e sinais clínicos e radiográficos em pacientes com infecção endodôntica.

No ensaio clínico randomizado, dentre os resultados obtidos nesta pesquisa, verificou-se que o PQM foi capaz de diminuir significativamente os níveis de carga microbiana total e de endotoxinas em dentes com infecções endodônticas primárias e lesão periapical. No entanto, mesmo obtendo uma redução significativa de carga bacteriana da ordem de 10⁵ para 10² UFC/mL, bactérias cultiváveis foram recuperadas de 50% dos canais radiculares nos tratamentos de sessão única. Esta limitação já havia sido demonstrado em estudos prévios (Xavier *et al.* 2013; Martinho *et al.*, 2014; Neves *et al.*, 2016). É importante relatar que maiores taxas de sucesso são encontrados em dentes com cultura bacteriana ausentes (Molander *et al.*, 2007; Sjögren *et al.*, 1997). Portanto, a persistência de bactérias cultiváveis no momento da obturação diminui o índice de sucesso (Sjögren *et al.*, 1997; Molander *et al.*, 2007).

Considerando que o PQM não é capaz de eliminar por completo microrganismos e seus subprodutos presentes nas infecções endodônticas, foi proposta um ensaio clínico randomizado utilizando a Terapia Fotodinâmica (PDT) como tratamento suplementar ao PQM. Seu mecanismo de ação é baseado na aplicação e retenção de um agente de fotossensibilização (FS) empregado em tecidos-alvo. Essa técnica utiliza espécies reativas de oxigênio (ROS), produzidos por moléculas desses corantes não-tóxicos ou FS na presença de luz visível (com comprimentos de onda específicos), após a irradiação com luz o FS sofre transição a partir de baixo nível de energia "estado fundamental" para um consumo de energia mais elevado. A ação do PDT é baseada no uso do agente fotossensibilizador com

luz de baixa energia para a produção de espécies radicais livres (Hamblin, Hasan, 2004) que é altamente reativo e é bem conhecido a agir em diferentes alvos de diferentes partes das bactérias, incluindo parede celular, DNA e membranas protéicas (George, Kishen, 2008). Tudo isto confere vantagens de ter atividade antimicrobiana de alto espectro e baixo risco de resistência.

A efetividade do PDT depende de diferentes aspectos, tais quais, agente de fotossensibilização, tempo de aplicação comprimento de onda, tipo de laser (Cherpa et al., 2014), e energia, tempo de emissão de laser, tipo de infecção bacteriana e modo de crescimento bacteriano (Upadya, Kishen, 2010).

Em relação ao agente de fotossensibilização, fenoatiazines e xantenos são os dois tipos comumente testados na eficácia antibacteriana (Soukos et al., 2006, Shrestha, Kishen, 2012). Foi selecionado o azul de metileno que pertence à categoria dos fenotiazinos e é efetivo contra diferentes bactérias como *Enterococcus* ssp e cepas resistentes à antibióticos (Wainwright, 1990; Soukos et al., 2006; George, Kishen, 2008). O alto efeito antibacteriano do azul de metileno é atribuído ao seu poder hidrofílico, baixo peso molecular, cationicidade (George, Kishen, 2008) e alta produção de oxigênio singlete (Shrestha, Kishen, 2012).

Particularidades do mecanismo de inativação bacteriana pelo azul de metileno foi previamente descrito na literatura (Wainwright et al., 1999), que é devido em grande parte ao corante fenotiazina (Wainwright et al., 1999; Tardivo et al., 2005).

De relevância clínica, nossos dados demonstraram que o PQM mais o PDT (aplicação de azul de metileno por 60 segundos exposto ao laser diodo com 660 nm de comprimento de onda e potência de 60 mW for 120 segundos) em sessão única reduziu consideravelmente o nível bacteriano (de 7.01×10^5 para 1.16×10^2 CFU/mL). Vale ainda ressaltar, que o NaOCl 2,5%, utilizado na presente pesquisa, apresenta alta atividade antimicrobiana e que a terapia com PDT na sessão única, aumentou o número de casos de cultura bacteriana negativa.

A suplementação da terapia endodôntica com PDT melhorou a descontaminação da carga microbiana total tanto na terapia de sessão única quanto múltipla. Entretanto, a terapia com PDT não foi capaz de melhorar a redução de endotoxinas alcançada com o PQM na sessão única e com a medicação à base de hidróxido de cálcio na sessão múltipla.

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APÊNDICE A – Detalhamento da metodologia

MATERIAL E MÉTODOS - Artigo 2

O projeto foi previamente enviado para o Comitê de Ética em Pesquisa (CEP) do Instituto de Ciências e Tecnologia de São José dos Campos – UNESP. Parecer 1321552 no Anexo C.

Seleção de pacientes

Para a realização da presente pesquisa foram selecionados 24 pacientes encaminhados para a Clínica de Endodontia do Departamento de Odontologia Restauradora da Faculdade de Odontologia de São José dos Campos (UNESP) com necessidade de tratamento endodôntico os quais 12 foram tratados em sessão única e 12 em sessão múltipla. Foi realizada anamnese e selecionado pacientes com condições favoráveis de saúde, local e sistêmica. Os pacientes assinaram um termo de consentimento livre e esclarecido (Anexo D), aceitando participar do estudo e também em cumprir as condições estabelecidas para a pesquisa.

Seleção de casos

Foram selecionados 24 pacientes com necessidade de tratamento endodôntico em dentes unirradiculares, com infecções endodônticas primárias. Foram excluídos pacientes que apresentaram qualquer alteração sistêmica; pacientes que fizeram uso de antifúngicos e antibióticos 3 meses antes do início da pesquisa. E ainda, foram excluídos da pesquisa dentes com dor espontânea, dentes com raízes curvas que impossibilitassem a inserção do cone por toda a extensão do canal radicular, dentes calcificados ou canais atrésicos que impossibilitassem a coleta com cone de papel, dentes com doença periodontal,

presença de fratura radicular, exposição da câmara pulpar ao meio bucal e impossibilidade de isolamento absoluto.

Etapas clínicas

Intervenção Odontológica

Todos os instrumentais utilizados nesta pesquisa foram submetidos à esterilização por radiação gama com cobalto 60 (dose 20 KGy por 6 horas) pela Embrarad (Empresa Brasileira de Radiação, Cotia, SP, Brasil) e todos os passos subsequentes foram realizados de forma asséptica. Inicialmente os pacientes foram submetidos à anestesia, isolamento absoluto seguido de profilaxia com pasta profilática (Vigodent S/A Indústria e Comércio, Rio de Janeiro, RJ, Brasil) e escova de Robinson (Microdont – Micro Usinagem de Precisão Ltda, São Paulo, SP, Brasil). Após, os dentes foram lavados com solução salina fisiológica (Laboratório Sanobiol, Pouso Alegre, MG, Brasil) e um vedamento da interface coroa/lençol foi feito utilizando uma barreira física de resina fotopolimerizável (Top Dam - FKG Dentaire – Labordental) (Fig. 1A, 1B).

Descontaminação do campo operatório

Previamente à coleta das amostras, foi realizada a desinfecção do campo operatório utilizando swabs (Jiangsu Medical Materials Co. Ltd., Jiangsu, China) estéreis umedecidos em água oxigenada 30 volumes (Byofórmula, São José dos Campos, SP, Brasil) seguido de hipoclorito de sódio 5,25% (Byofórmula, São José dos Campos, SP, Brasil). Foi realizada abertura coronária com auxílio de brocas acionadas em alta rotação, sob refrigeração com solução salina fisiológica. Foi realizada neutralização do conteúdo séptico do canal radicular e posteriormente, preparo biomecânico utilizando hipoclorito de sódio 2,5% [45]. A esterilidade do campo operatório após o protocolo de desinfecção

foi checada por coletas realizadas da coroa dental antes da abertura coronária (Fig. 1C).

Procedimentos endodônticos

A fase de acesso coronário foi realizada em duas etapas operatórias. A água proveniente do equipo foi cessada, sendo a irrigação realizada manualmente com solução salina estéril. Brocas de alta rotação diamantadas estéreis/ apirogênicas (esterilizadas através de calor seco, em estufa por um período de tempo de 4 horas a 200°C) foram utilizadas. Na primeira etapa operatória foi realizada a remoção dos contaminantes coronários (restaurações, tecido cariado, microrganismos). Na segunda etapa, na fase de confecção da cavidade de acesso, nova broca estéril/ apirogênica foi utilizada. Após a confecção da cavidade de acesso, nova desinfecção foi realizada prosseguindo com o completo acesso ao canal radicular, com nova broca esférica diamantada estéril/ apirogênica (Fig. 1D, 1E).

Momentos das coletas clínicas - Coleta de Endotoxinas (CE)

Grupo Sessão única:

1. Coleta de endotoxinas inicial (CESU1)
2. Coleta de endotoxinas após preparo químico-mecânico (CESU2)
3. Coleta de endotoxinas após EDTA (CESU3)
4. Coleta de endotoxinas após terapia fotodinâmica (CESU4)

Grupo Sessão múltipla:

1. Coleta de endotoxinas inicial (CESM1)
2. Coleta de endotoxinas após preparo químico-mecânico (CESM2)
3. Coleta de endotoxinas após EDTA (CESM3)
4. Coleta de endotoxinas após medicação intracanal (CESM4)
5. Coleta de endotoxinas após terapia fotodinâmica (CESM5)

Momentos das coletas clínicas- Coleta Microbiológica (CM)

Grupo Sessão única:

1. Coleta microbiológica inicial (CMSU1)
2. Coleta microbiológica após preparo químico-mecânico (CMSU2)
3. Coleta microbiológica após EDTA (CMSU3)
4. Coleta microbiológica após terapia fotodinâmica (CMSU4)

Grupo Sessão múltipla:

1. Coleta microbiológica inicial (CMSM1)
2. Coleta microbiológica após preparo químico-mecânico (CMSM2)
3. Coleta de endotoxinas após EDTA (CMSM3)
4. Coleta microbiológica após medicação intracanal (CESU4)
5. Coleta microbiológica após terapia fotodinâmica (CMSM5)

Coleta microbiológica (CM) e endotoxinas inicial (CE)

Para a coleta de amostras microbiológicas (CMSU1 e CMSM1) do canal radicular antes do preparo químico mecânico (Fig.1- F), 3 cones de papel absorventes (Dentsply Maillefer Ind. E Com. Ltda, Petrópolis, RJ, Brasil) apirogênicos foram introduzidos um de cada vez no interior do canal radicular até próximo ao comprimento total do canal radicular (determinado pela radiografia pré-operatória) permanecendo nesta posição por 60 segundos; em seguida, foram transferidos para tubo tipo eppendorf (1,5 mL) esterilizado contendo 1 mL de meio de transporte pré-reduzido VMGA III - Viability Medium Goteberg Agar [46, 47] para posterior análise microbiológica. Para a coleta de endotoxinas (CE), um cone de papel estéril/ apirogênico foi introduzido no canal, e deixado por 60 segundos, e em seguida armazenado em

tubo tipo eppendorf (1,5mL) estéril/ apirogênico por radiação gama com cobalto para quantificação de endotoxinas através do teste de endotoxinas.

Após, a coleta inicial dos canais radiculares, os 24 dentes com infecção endodôntica primária foram divididos aleatoriamente em 2 grupos: SU - sessão única (n=12) - que foram submetidos ao PQM, EDTA 17% e aplicação de PDT; e SM – sessão múltipla (=12) que foram submetidos ao PQM, EDTA 17%, utilização de medicação intracanal e aplicação de PDT.

Instrumentação do canal radicular – Preparo Químico-mecânico (PQM)

Após a coleta inicial, o comprimento de trabalho (CT) foi determinado radiograficamente e confirmado com o uso do localizador foraminal (*RomiApex A-15; Romidan Dental Solution, Kiryat-Ono, Israel*). O canal radicular foi instrumentado por toda sua extensão, considerando o CT o “ponto 0”, determinado no localizador foraminal.

Como preconizado pelo fabricante do sistema Reciproc® (VDW, Munique, Alemanha) utilizou-se apenas uma lima do sistema recíprocante em cada caso. Quando o diâmetro anatômico do canal permitiu passivamente a entrada de um instrumento tipo K # 30 até o comprimento de trabalho, utilizou-se a lima R 50. Se a lima tipo K # 20 foi o instrumento de máximo diâmetro que atingiu o CT de maneira passiva, empregou-se a lima R 40. Se a lima tipo K # 20 não conseguiu chegar ao CT, a lima de eleição foi a R 25.

Nos casos em que, após a instrumentação com o sistema recíprocante, alcançou 2/3 do CT e a lima tipo K # 10 não atingiu passivamente o CT, como o fabricante recomenda, foi feito o pathfile até a lima tipo K # 15 antes de se concluir com a lima R 25 a preparação final até o CT. Entende-se por passivo, que a lima por meio de movimentos de pequenas amplitudes e pequena pressão digital e sem realizar rotações, mas oscilando-se para a esquerda e para a direita, possa chegar ao comprimento de trabalho.

Durante todo o preparo, o canal foi irrigado com 10 mL de hipoclorito de sódio 2,5%. Ao final do preparo químico mecânico utilizou-se 5 mL de Tiosulfato de sódio para neutralização do hipoclorito de sódio [45] e então o canal foi lavado com 10 mL de soro fisiológico estéril/ apirogênico.

Coleta microbiológica e de endotoxinas após PQM e irrigação com EDTA

As coletas microbiológicas (CMSU2 e CMSM2) e de endotoxinas (CESU2 e CESM2) após PQM foram realizadas de acordo com a coleta inicial previamente descrita (Fig. 1-i). Após a segunda coleta, os canais foram irrigados com EDTA 17% por 3 minutos, seguido de irrigação final com 10 mL de solução salina fisiológica estéril/apirogênica. Após isso, os grupos foram submetidos à terceira coleta microbiológica (CMSU3 e CMSM3) e de endotoxinas (CESU3 e CESM3).

Os dentes do grupo da sessão múltipla foram então preenchidos com medicação à base de hidróxido de cálcio $[Ca(OH)_2]$ + Solução Salina [SSL], preparada na proporção 1:1 em volume e levadas ao canal radicular com o auxílio de limas e lentulo (Dentsply/Maillefer Instruments SA, Ballaigues, Swistzerland). Em seguida, os dentes foram selados com uma camada de coltosol (Vigodent, Rio de Janeiro, RJ, Brasil) seguido de restauração provisória com cimento de ionômero de vidro (S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brasil), os quais permaneceram com medicação pelo período de 14 dias.

Coleta microbiológica e de endotoxinas após medicação intracanal (CMI)

Transcorrido o período de 14 dias, o paciente foi novamente submetido à anestesia e isolamento absoluto seguido de profilaxia. Após, os dentes foram lavados com solução salina fisiológica e um vedamento da interface coroa/lençol foi realizado utilizando uma barreira física de resina fotopolimerizável (Top

Dam - FKG Dentaire – Suíça). Em seguida, foi realizada a remoção do selamento coronário, remoção da medicação intracanal (MIC) com lima K #30 (Dentsply/Maillefer Instruments SA, Ballaigues, Switzerland) e irrigação com 20 mL de solução salina fisiológica apirogênica. Após a remoção da MIC, novas coletas microbiológica (CMSM4) e de endotoxinas (CESM4) foram realizadas de acordo com as coletas previamente descritas.

Aplicação da Terapia Fotodinâmica (PDT)

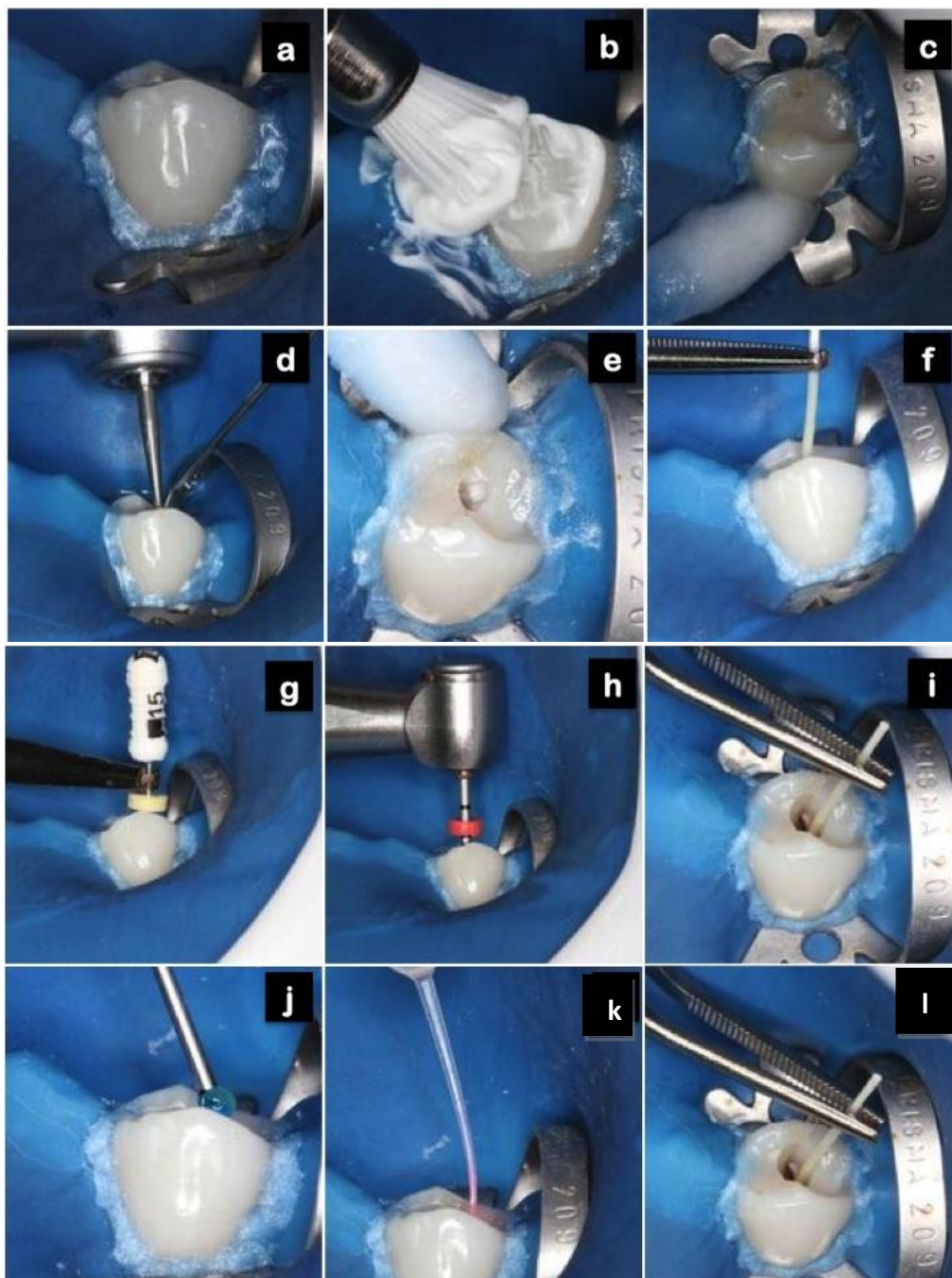
Na sequência, os canais da sessão única e múltipla receberam o protocolo de PDT para auxiliar na descontaminação do canal radicular. Para tal, foi aplicado o fotossensibilizador (azul de metileno 10mg/ml) no canal previamente preparado. Após um minuto, o canal foi lavado com água e exposto ao laser de baixa potência (Thera Lase DMC – São Carlos, SP- Brasil), com uma fibra ótica correspondente, de diâmetro de 600 µm, que foi aplicada em todo o comprimento do canal. Foi utilizado um comprimento de onda de 660 nm, com potência de 60 mW e uma dose de 129 J/cm², durante um minuto (Fig1 - j, k).

Coleta microbiológica e endotoxinas após terapia fotodinâmica

As coletas microbiológicas (CMSU4 e CMSM5) e de endotoxinas (CESU4 e CESM5) após terapia fotodinâmica foram realizadas de acordo com as coletas previamente descritas (Fig1- l).

Seguido às últimas coletas, os dentes dos grupos de sessão única e múltipla foram obturados.

Figura 1- Procedimentos clínicos e coletas endodônticas



Legenda: a) Isolamento absoluto; b) profilaxia; c) descontaminação do campo operatório; d) abertura coronária sob irrigação com soro fisiológico; e) visualização da primeira etapa operatória e nova desinfecção do campo operatório; f) coleta microbiológica e de endotoxinas; g) odontometria eletrônica; h) instrumentação mecânica Reciproc; i) coleta pós PQM; j) aplicação do fotossensibilizador (azul de metileno) no canal radicular; k) aplicação do laser (PDT); l) coleta pós PDT.

Processamento microbiológico – Contagem de UFC

Para todas as coletas, a atividade antimicrobiana foi avaliada pela contagem de unidades formadoras de colônias por mililitro (UFC/mL) de microrganismos remanescentes no canal radicular. O eppendorf que continha os 3 cones de papel (amostra matriz), armazenados em 1 mL de meio de transporte pré-reduzido VMGA III, foram agitados até a dessorção do conteúdo para facilitar a dispersão dos microrganismos. Em seguida, foram realizadas diluições seriadas transferindo 100 μ L da amostra matriz para a diluição 10^{-1} e assim consecutivamente até a diluição 10^{-3} em tubos eppendorf (1,5 mL) contendo 900 μ L de Caldo Infuso Cérebro Coração (BHI) (Brain Heart Infusion Broth – HiMedia Laboratories Pvt. Ltda, Mubai, Índia).

Em seguida, foram semeados 50 μ L da amostra matriz (sem diluição) e 50 μ L da diluição 10^{-3} em placas de Petri (Interlab - São Paulo, SP, Brasil) contendo Fastidious Anaerobe Agar (FAA) (Neogen Corporation, Michigan, USA), acrescido de 5% de sangue de carneiro desfibrinado suplementados com hemina (5 mg/L) e vitamina K1 (1 mg/L). Essas placas foram incubadas em jarra de anaerobiose a 37 °C por 14 dias, para a detecção de microrganismos anaeróbios e facultativos. O número de unidades formadoras de colônias (UFCs) foi determinado a partir da contagem do número de colônias nas placas analisadas levando em consideração a diluição das amostras para obtenção do cálculo final de UFCs.

Quantificação de endotoxinas

Para quantificação de endotoxinas presentes nas amostradas coletadas dos canais radiculares foi utilizado o método cromogênico cintético: teste KQCL (Lonza, Walkersville, MD, EUA) - uma preparação de Lisado de Amebócito *Limulus* (LAL) [48].

Extração de endotoxinas do cone de papel:

Para extração de endotoxinas, as amostras coletadas dos canais radiculares foram reconstituídas em 1 mL de água de LAL e em seguida extraídas sob agitação mecânica em vortex por 60 segundos.

Figura 2 - Quantificação de endotoxina



Legenda: a) extração de endotoxina em vortex por 60 s; b) água de LAL; c) Placas de 96 poços (Costar); d) reagentes Kit KQCL; e) inoculação das amostras, padrões, blank e substrato de LAL; f) leitor de microplacas.

Teste KQCL para quantificação de endotoxinas LAL

Para verificação dos níveis de endotoxinas, foi utilizado o teste cinético cromogênico do lisado de amebócitos de *Limulus* (análise quantitativa) (Lonza, Walkersville, MD, EUA). Em uma placa apirogênica de 96 poços, foram adicionados 100 μ L de água apirogênica (branco da reação), os padrões de

endotoxina, as amostras coletadas do canal radicular e os controles positivos. A placa foi incubada no leitor cinético QCL a 37 ± 1 °C por 10 minutos, o qual estava acoplado a um microcomputador com software WinkQCL™ específico para gerenciamento, execução e emissão de relatórios. Após, foram adicionados em cada poço da placa 100 µL do reagente cinético cromogênico do LAL, com uma micropipeta multicanal e ponteiros apirrogênicos. Após o início do ensaio cinético, o software da leitora de microplacas monitorava, de forma contínua durante todo o ensaio, a absorbância a 405 nm em cada poço da microplaca. O leitor determinou o tempo necessário para aumentar a absorbância de cada poço a 0.200 unidades de absorbância, que foi denominado tempo de reação.

Procedimento para realização do teste Pyrogent-5000

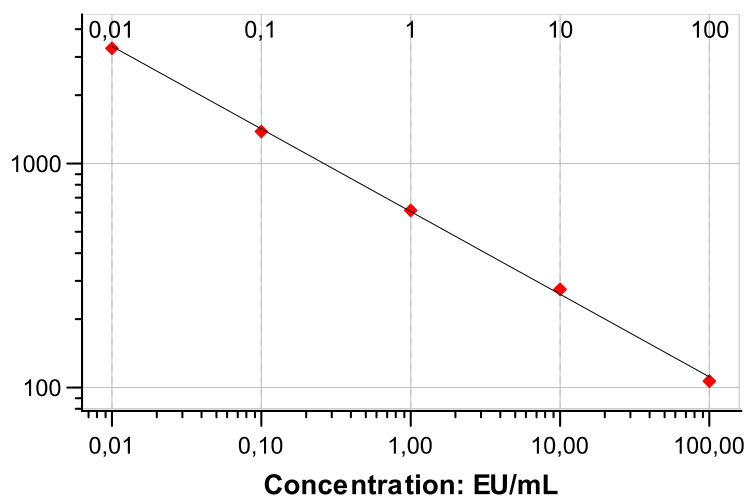
Inicialmente, 100 µL do “blank” (água de LAL) foi inoculado em duplicata na placa de 96 poços (Corning Costar, Cambridge, MA) (Figura 3). Em seguida, 100 µL de cada ponto da curva padrão nas diferentes concentrações (50 EU/mL, 5 EU/mL, 0,5 EU/mL, 0,05 EU/mL) foi distribuído em duplicata (Figura 2). Após, 100 µL das amostras e seus respectivos controles (PPC) - ambos em duplicata foram inoculados com reagente de LAL (Figura 3). Para cada poço do controle positivo foi adicionado 10 µL de 0,1 EU/mL de endotoxina de *E. coli* (spike). A placa já posicionada no leitor de ELISA (Ultramark, Bio-Rad Laboratories) com Software WinKQCL® e programado para - 340 nm, 37 ° C e 100 leituras - 100 µL do reagente de LAL foi adicionado em todos os poços. Em seguida, a leitura foi iniciada, levando aproximadamente 60 min para o término do teste.

Figura 3 - Distribuição das amostras

	A	B	C	D	E	F	G	H	I	J	K	L
1	B	B	A	A	A	A	A	A	A	A	A	A
2	D1	D1	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC
3	D2	D2	A	A	A	A	A	A	A	A	A	A
4	D3	D3	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC
5	D4	D4	A	A	A	A	A	A	A	A	A	A
6	D5	D5	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC
7	A	A	A	A	A	A	A	A	A	A	A	A
8	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC	AC

Legendas: B= blank; D1= 50 EU/mL, D2= 5 EU/mL; D3= 0,5 EU/mL; D4= 0,05EU/mL; A= Amostra; AC= Amostra Controle.

Figura 4 - Curva padrão gerada pelo teste KQCL para determinação da



concentração de endotoxinas presente nas amostras endodônticas

Os dados coletados foram introduzidos numa planilha de cálculo e submetidos à análise estatística com o SPSS for Windows (SPSS Inc., Chicago,

Illinois, USA). Os testes T-student, Qui-quadrado e Wilcoxon foram utilizados para comparar os resultados obtidos. Foram comparados os níveis microbiológicos e de endotoxinas, nos diferentes tempos, num mesmo paciente e entre diferentes pacientes analisados, com nível de significância de 5%.

ANEXO A – Comprovante de artigo submetido ao Periódico *International Endodontic Journal*

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ScholarOne Manuscripts™ Frederico Martinho Instructions & Forms Help

International Endodontic Journal
The Official Journal of the British Endodontic Society and the European Society of Endodontology

Home Author

Author Dashboard / Submission Confirmation

Submission Confirmation

Thank you for your submission

Submitted to	International Endodontic Journal
Manuscript ID	IEJ-16-00526
Title	Does supplemental photodynamic therapy optimize the disinfection of bacteria and endotoxins in one-visit and two-visit root canal therapy? A randomised clinical trial
Authors	Martinho, Frederico Corazza, Bruna Ferreira, Luciana Rabello, Diego Santamaria, Mauro Gomes, Ana Paula
Date Submitted	08-Aug-2016

ANEXO B – Comprovante de artigo submetido ao Periódico *Journal of Endodontics*

Dr Frederico C. Martinho
São José dos Campos Dental School
Endodontic Division
Av. Francisco José Longo, 777 Bairro São Dimas
São José dos Campos, São Paulo – Brazil.
12245-000 - Phone: (55) 12 – 39479050
email: Frederico.martinho@fosjc.unesp.br

3rd August 2016

Journal of Endodontics
Kenneth Hargreaves, DDS, PhD
Editor, Dept. of Endodontics, UTHSCSA
7703 Floyd Curl Dr, San Antonio, TX 78 229-3900
Fax: 210 / 567-3389
E-mail: JEndodontics@UTHSCSA

Dear Professor Hargreaves,

We are sending an original scientific article entitled **"ENDOTOXIN IN ROOT CANAL INFECTION: A SYSTEMATIC REVIEW AND META-REGRESSION ANALYSIS"** for publication appreciation in Journal of Endodontics. This manuscript represents unpublished original research that is not being considered for publication elsewhere. In consideration of the editors of the *Journal of Endodontics* taking action in reviewing and editing this submission, the authors undersigned hereby transfer, assign or otherwise convey all copyright ownership to the AAE in the event that such work is published in that Journal. We affirm that we have no financial affiliation (e.g., employment, direct payment, stock holdings, retainers, consultancies, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest is disclosed. The informed consent of all human subjects who participated in the experimental investigation reported or described in this manuscript was obtained after the nature of the procedure and possible discomforts and risks had been fully explained.

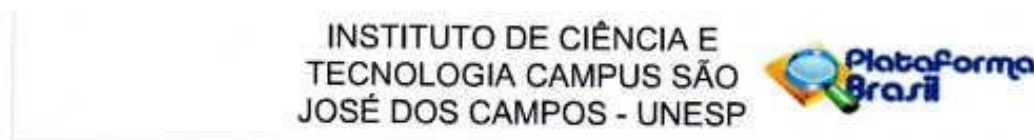

Frederico Canato Martinho


Diego Guilherme Dias de Rabello


Luciana Louzada Ferreira


Gustavo G. Nascimento

ANEXO C – Certificado do Comitê de Ética em Pesquisa



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO CLÍNICA DO EFEITO DO PDT NA SUPLEMENTAÇÃO DA DESINFECÇÃO DE CANAIS RADICULARES TRATADOS NAS MODALIDADES DE TERAPIA ENDODÔNTICA EM ÚNICA E MÚLTIPLAS SESSÕES

Pesquisador: FREDERICO CANATO MARTINHO

Área Temática:

Versão: 4

CAAE: 43555515.9.0000.0077

Instituição Proponente: Instituto de Ciência e Tecnologia de São José dos Campos - UNESP

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.321.552

Apresentação do Projeto:

Diferentes são as modalidades de terapia endodôntica nos dias atuais, podendo ser realizada em única ou múltiplas sessões. Sabe-se que o preparo químico-mecânico (PQM) não é capaz de eliminar por completo microrganismos presentes nas infecções Endodônticas. Desta forma, a busca por um protocolo suplementar ao PQM têm sido alvo na Endodontia. Particularmente a Terapia Fotodinâmica (PDT), chama a atenção pois já foi utilizado como tratamento para várias doenças.

Objetivo da Pesquisa:

Os objetivos do presente estudo são:

- 1) Avaliar e comparar a efetividade da suplementação da terapia endodôntica com o uso de PDT na redução microbiana presente nas infecções endodônticas primárias nas modalidades de terapia endodôntica em única e múltiplas sessões;
- 2) Investigar a efetividade do PDT na redução de endotoxinas presente nas infecções Endodônticas primárias nas modalidades de terapia endodôntica em única e múltiplas sessões.

Avaliação dos Riscos e Benefícios:

De acordo com o pesquisador, o projeto não acarretará risco algum para o paciente uma vez que

Endereço: Av. Engº Francisco José Longo 777

Bairro: Jardim São Dêmas

CEP: 12.245-000

UF: SP

Município: SÃO JOSÉ DOS CAMPOS

Telefone: (12)3947-8078

Fax: (12)3947-8010

E-mail: ceph@fcsjc.unesp.br

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Continuação do Parecer: 1.321.552

de acordo com o pesquisador, o projeto não acarretará risco algum para o paciente uma vez que será realizado dentro das normas de biossegurança. Além disso, o protocolo de conduta adotado é o mesmo já utilizado na prática clínica odontológica desta faculdade.

Benefícios:

Contribuição para maior respaldo do uso do PDT na desinfecção dos canais radiculares; apresentar e validar protocolo clínico à comunidade de endodontia, responder aos clínicos se seu uso justifica-se tanto nas modalidades de terapia endodôntica realizadas em única e múltiplas sessões. É importante ressaltar que nenhum estudo até o momento avaliou a efetividade da terapia fotodinâmica (PDT) sobre endotoxinas presente nas infecções endodônticas, as quais apresentam alto potencial inflamatório para os tecidos periapicais. Sendo assim este trabalho permitirá gerar dados inéditos ainda não reportados na literatura endodôntica.

Comentários e Considerações sobre a Pesquisa:

A pesquisa se propõe a comprovar procedimentos importantes e inéditos na área da endodontia. Esperamos que os resultados esperados sejam atingidos e sejam divulgados para a comunidade clínica a fim de proporcionar melhor qualidade no atendimento aos pacientes.

Considerações sobre os Termos de apresentação obrigatória:

Os termos obrigatórios foram apresentados e a pendência anterior sobre a adequação do TCLE foi atendida, ou seja, o pesquisador incluiu os objetivos da pesquisa, a metodologia a ser utilizada, as etapas da pesquisa e os resultados esperados. A utilização de linguagem acessível e de fácil compreensão aos participantes da pesquisa também foi utilizada.

Recomendações:

A recomendação anterior também foi atendida.

Conclusões ou Pendências e Lista de Inadequações:

Não há pendências.

Considerações Finais a critério do CEP:

O Colegiado acata o parecer da relatora.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações	PB_INFORMAÇÕES_BÁSICAS_DO_P	04/11/2015		Aceito

Endereço: Av. Engº Francisco José Longo 777

Bairro: Jardim São Dimas

CEP: 12.245-000

UF: SP

Município: SÃO JOSÉ DOS CAMPOS

Telefone: (12)3947-9078

Fax: (12)3947-9010

E-mail: cep@fosjc.unesp.br

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Continuação do Parecer: 1.321.552

Básicas do Projeto	ETO_485711.pdf	12:17:08		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	04/11/2015 12:16:35	FREDERICO CANATO MARTINHO	Aceito
Projeto Detalhado / Brochura Investigador	ProjetoICBrunaOficial.doc	04/11/2015 12:16:13	FREDERICO CANATO MARTINHO	Aceito
Folha de Rosto	Folha de rosto bruna .pdf	29/03/2015 11:44:09		Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

SAO JOSE DOS CAMPOS, 13 de Novembro de 2015

Assinado por:
Denise Nicodemo
(Coordenador)

Endereço: Av. Engº Francisco José Longo 777

Bairro: Jardim São Dimas

CEP: 12.245-000

UF: SP

Município: SÃO JOSE DOS CAMPOS

Telefone: (12)3947-9078

Fax: (12)3947-9010

E-mail: cep@fosjc.unesp.br

ANEXO D - Termo de Consentimento Livre e Esclarecido

Caro(a) Senhor(a)



Eu, Frederico Canato Martinho, professor da Disciplina de Endodontia da Faculdade de Odontologia de São José dos Campos - UNESP, portadora do CPF 092.585.227.96, estabelecido à Avenida Eng. Francisco José Longo, 777, na cidade de São José dos Campos-SP, telefone para contato (12) 3947 9048, sou responsável pela pesquisa cujo título é **“AVALIAÇÃO CLÍNICA DO EFEITO DO PDT NA SUPLEMENTAÇÃO DA DESINFECÇÃO DE CANAIS RADICULARES TRATADOS NAS MODALIDADES DE TERAPIA ENDODÔNTICA EM ÚNICA E MÚLTIPLAS SESSÕES”**.

O objetivo deste estudo é avaliar e comparar a efetividade da suplementação da terapia endodôntica com o uso de PDT na redução microbiana presente nas infecções endodônticas primárias nas modalidades de terapia endodôntica em única e múltiplas sessões; Além disso tem por objetivo investigar a efetividade do PDT na redução de endotoxinas presente nas infecções endodônticas primárias nas modalidades de terapia endodôntica em única e múltiplas sessões.

O projeto não acarretará risco algum para o paciente uma vez que será realizado dentro das normas de biossegurança. Além disso, o protocolo de conduta adotado é o mesmo já utilizado na prática clínica odontológica desta faculdade. O paciente receberá número de telefone para entrar em contato em caso de dor e será atendido imediatamente. Os pacientes serão chamados para acompanhamento radiográfico após 1 ano do tratamento finalizado.

Se houver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética (CEP) da Faculdade de Odontologia de São José dos Campos - UNESP, situada na Av. Engº Francisco José Longo, 777 – CEP 12245000, em São José dos Campos-SP. Informo que será garantida a liberdade da retirada do consentimento a qualquer momento e assim deixar de participar do estudo. Também não haverá custo nem pagamento pela colaboração.

Termo de Consentimento Livre e Esclarecido

Acredito ter sido esclarecido(a) a respeito das informações que leram para mim, descrevendo o estudo a ser realizado e concordo em receber atendimento em consultório odontológico. Declaro conhecer os propósitos do estudo, os procedimentos a serem realizados, as garantias de confidencialidade e de esclarecimentos permanentes, e que minha participação não implicará em nenhuma despesa.

NOME/RESPONSÁVEL _____

RG _____ CPF _____

ENDEREÇO COMPLETO _____

Assinatura Responsável

Assinatura Pesquisador