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ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA – ENDODONTIA

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TERAPIA FOTODINÂMICA REDUZ A CARGA MICROBIANA EM DENTES COM INFECÇÃO ENDODÔNTICA PRIMÁRIA? UMA REVISÃO SISTEMATICA DA LITERATURA E META-ANÁLISE

Porto Alegre
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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Odontologia da Universidade Federal do Rio Grande do Sul, como pré-requisito final para a obtenção do título de Doutor em Clínica Odontológica – Endodontia.

Linha de Pesquisa: Biomateriais e Técnicas Terapêuticas em Odontologia.

Orientador: Profª. Drª. Patrícia Maria Poli Kopper Móra

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RESUMO

Introdução: A terapia fotodinâmica antimicrobiana (aPDT) consiste na utilização de um corante fotossensível (PS) e uma fonte de luz como auxiliar na desinfecção após o preparo químico-mecânico dos canais. O objetivo dessa revisão sistemática foi avaliar a capacidade da aPDT em aumentar a desinfecção proporcionada pelo preparo químico-mecânico. **Metodologia:** Uma busca sistematizada da literatura foi realizada em 4 bases de dados online (PubMed, EMBASE, Web Of Science, Scopus) até agosto/2020. Ensaios clínicos e ensaios clínicos randomizados em que os participantes apresentavam infecção endodôntica primária em dentes permanentes e que a compararam a carga microbiana antes e depois da aPDT foram incluídos. Dois pesquisadores independentes avaliaram os títulos e os resumos para definir os estudos elegíveis. O risco de viés e qualidade metodológica foram avaliados com as ferramentas RoB2, ROBINS-I, CONSORT e TREND. Estudos com texto completo indisponível foram excluídos. A análise dos dados foi realizada com teste de Egger, I^2 e meta-análise a partir do software R. A revisão foi registrada no PROSPERO (CRD42020181783). **Resultados:** Dos 3625 estudos localizados na busca, sete foram incluídos na análise qualitativa e cinco foram elegíveis para a meta-análise. No modelo de efeito fixo, aPDT melhorou significativamente e desinfecção do canal radicular em comparação com o preparo químico-mecânico tradicional ($P<0,05$). A análise de subgrupo indica que a aPDT tem um efeito maior em microorganismos anaeróbios. **Conclusões:** O presente estudo mostrou que a aPDT é uma terapia adicional eficaz no combate à infecção quando usada após o preparo químico-mecânico. Os dados apresentados sugerem que aPDT melhora a capacidade de desinfecção de tratamentos endodônticos de dentes apresentando infecção endodôntica primária.

Palavras-Chave: Endodontia, Desinfecção do Canal Radicular, Terapia Fotodinâmica Antimicrobiana

ABSTRACT

Introduction: Photodynamic therapy (aPDT) consists in using a photosensitizer (PS) and a light source as a supplementary method in root canal treatment after the chemo-mechanical disinfection. The aim of this systematic review was to evaluate whether aPDT yields a greater microbial load reduction than chemo-mechanical root canal disinfection. **Methods:** An electronic literature search was conducted on four databases (PubMed, EMBASE, Web Of Science, Scopus) until august/2020 with no language or publication period restrictions. Clinical trials and randomized clinical trials in which participants presented primary endodontic infection in permanent teeth and which compared microbial load before and after using aPDT were included. Two researchers independently screened the title and abstract to define studies eligibility. Risk of bias and methodological quality of the studies were accessed with RoB2, ROBINS-I, CONSORT and TREND tools. Studies with full text unavailable were excluded. Egger test, i^2 and meta-analysis were conducted in R software. The review was registered in PROSPERO (CRD42020181783). **Results:** Seven studies were included in qualitative analysis, and five were eligible for meta-analysis. In the fixed effect model, aPDT significantly enhanced root canal disinfection compared to standard protocols for cleaning and shaping ($p<0.05$). Subgroup analysis indicate that aPDT presents higher effect on decreasing anaerobes microorganisms' load. **Conclusions:** The present study shows that the use of aPDT as an adjunct of chemo-mechanical disinfection is effective in promoting additional reduction of endodontic microbial load. The compilated data suggests that aPDT should help to improve the results of root canal treatments in permanent teeth with primary endodontic infection.

Keywords: Antimicrobial Photodynamic Therapy, Root Canal Disinfection, Endodontics

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1 APRESENTAÇÃO

A presente tese teve como foco de estudo avaliar o efeito do aPDT na redução da carga microbiana intracanal após uma desinfecção convencional feita pelo preparo químico mecânico dos canais radiculares. Dessa forma foram avaliados estudos que fizeram análise quantitativa de microorganismos antes e após o emprego do aPDT em dentes que apresentavam infecção endodôntica primária.

Essa tese está estruturada da seguinte maneira:

- Introdução, enfatizando os pontos principais do estudo e apresentando uma revisão de literatura do assunto da tese;

- Artigo Científico: o desenvolvimento do trabalho está apresentado na forma de artigo cientifico de periódico em inglês, a ser enviado para publicação na revista Journal of Endodontics, fator de impacto 4.422 (Qualis A1, CAPES);

- Considerações finais.

2 INTRODUÇÃO

Os microrganismos são os principais agentes etiológicos das patologias pulpares e periapicais (KAKEHASHI; STANLEY; FITZGERALD, 1965). Por esse motivo, o tratamento endodôntico visa uma desinfecção do sistema de canais radiculares previamente a uma obturação tridimensional e selamento da cavidade pulpar. Uma revisão de literatura de 2010 (NG; MANN; GULABIVALA, 2010), relatou que o tratamento endodôntico tem taxas de sucesso entre 86% e 93%. Entretanto, quando os parâmetros de qualidade técnica do tratamento não são atingidos, a chance de insucesso aumenta (BYSTRÖM; SUNDQVIST, 1981; SIQUEIRA; RÔÇAS, 2008; SJÖGREN *et al.*, 1997; SUNDQVIST *et al.*, 1998).

Para que seja possível a correta desinfecção do sistema de canais radiculares, primeiro é necessário o entendimento das características e classificações dessa microbiota. Pode ser dividida pela localização, intra e extra radicular, além de classificada em primária e secundária ou persistente (LOPES; SIQUEIRA, 2015). A infecção endodôntica primária é polimicrobiana, possuindo em média 10 a 30 espécies, contendo 10^3 e 10^8 células bacterianas por canal. Tem predomínio de anaeróbias estritas gram positivas e negativas, com grande variação de acordo com o tempo de infecção, tipo de resposta inflamatória e tamanho da lesão apical (LOPES; SIQUEIRA, 2015; SIQUEIRA; RÔÇAS, 2005, 2009; SIQUEIRA; RÔÇAS; LOPES, 2002).

Uma vez instalada na câmara pulpar, a infecção pode se espalhar e contaminar áreas adjacentes como canais radiculares, istmos, canais laterais, acessórios e túbulos dentinários, onde os microrganismos são capazes de penetrar 1250µm (SIQUEIRA; RÔÇAS; LOPES, 2002; VERA *et al.*, 2012). Vera e colaboradores (VERA *et al.*, 2012), em um estudo comparando a desinfecção obtida em uma única consulta com a obtida em duas consultas com emprego de medicação intracanal, teve como resultado que todos os dentes que foram tratados em uma única consulta, tinham infecção persistente. Por outro lado, 71% de dentes tratados em duas consultas apresentaram infecção persistente. Áreas de maior complexidade anatômica, como istmos e deltas apicais, estão associadas com maior carga microbiana (VERA *et al.*, 2012).

O tratamento endodôntico convencional (CD), composto por instrumentação mecânica e irrigação com substancias químicas auxiliares é insuficiente para a remoção completa da infecção (LIN *et al.*, 1991; SIQUEIRA *et al.*, 1997; VERA *et al.*, 2012). A partir disso, métodos de desinfecção auxiliares vêm sendo propostos, sendo o emprego de uma medicação intracanal o mais conhecido e difundido. Consiste num fármaco aplicado dentro do espaço pulpar, sendo a pasta de hidróxido de cálcio o mais utilizado (LOPES; SIQUEIRA, 2015).

A pasta de hidróxido de cálcio é utilizada com diversos veículos com propriedades distintas, porém sempre atua por contato e pela liberação de íons hidroxila (OH^-), trazendo a alcalinidade ao meio (referência). Deve ser utilizada entre consultas com intervalo de pelo menos 1 semana (MOHAMMADI; DUMMER, 2011). A atividade antimicrobiana do hidróxido de cálcio atua causando danos à membrana plasmática e ao DNA das células bacterianas (MOHAMMADI; DUMMER, 2011). Além disso o hidróxido de cálcio é a única medicação intracanal capaz de inativar o LPS bacteriano, que é uma endotoxina presente na parede celular bacteriana capaz de estimular resposta inflamatória periapical (LEONARDO *et al.*, 2004). Porém, o emprego da pasta de hidróxido de cálcio para potencializar a desinfecção do sistema de canais após a conclusão do preparo químico mecânico tem sido questionada. Estudos que avaliaram o sucesso do tratamento endodôntico não encontraram diferenças significativas quando comparados tratamentos com e sem o emprego da medicação (MOLANDER *et al.,* 2007; MOREIRA *et al.,* 2017). Já quando a resposta histológica foi avaliada após a conclusão do tratamento endodôntico, um melhor reparo quando a pasta de hidróxido de cálcio foi empregada (LEONARDO *et al.,* 2006)

Frente a essas resultados contraditórios e a necessidade de recursos que possam potencializar a desinfeção do sistema de canais obtida após a conclusão do preparo químico mecânico, a terapia fotodinâmica antimicrobiana (aPDT), vem ganhando espaço. Essa técnica visa a diminuição da carga microbiana no interior do canal radicular com a utilização de um corante fotoativo (fotossensibilizador) e uma fonte específica de emissão de luz (GURSOY *et al.*, 2013; LOPES; SIQUEIRA, 2015; PLOTINO; GRANDE; MERCADE, 2019).

O mecanismo de ação da aPDT se dá pela capacidade do fotossensibilizador (PS) de ser excitado por uma luz de comprimento de onda específico e produz uma resposta fototóxica na presença do oxigênio (CARRERA *et al.*, 2016; GURSOY *et al.*, 2013; PLOTINO; GRANDE; MERCADE, 2019). A transferência de energia do PS ativado para o oxigênio disponível no ambiente resulta na formação de compostos como o oxigênio *singlet* e os radicais livres, os quais são moléculas altamente reativas (LOPES; SIQUEIRA, 2015). O oxigênio do ar atmosférico está em estado *triplet*, o qual não está muito reativo, se ele absorver energia suficiente, pode se tornar um *singlet* e, dessa forma, ter atividade antimicrobiana (LOPES; SIQUEIRA, 2015). Também chamados de espécies reativas de oxigênio (ROS) elas podem modificar as estruturas da membrana plasmática, o DNA, causar peroxidação lipídica, inibição enzimática e aglutinação de proteínas. Durante a aPDT apenas as células que estão expostas ao corante e à luz são atingidas, além disso, a ausência de efeitos genotóxicos e mutagênicos da aPDT sobre os tecidos do hospedeiro é um fator importante de segurança do tratamento (CARRERA *et al.*, 2016; KONOPKA; GOSLINSKI, 2007).

As características ideais de um PS são: ausência de toxicidade, ausência de mutagenicidade, acumulação seletiva no tecido alvo, aplicação tópica, baixo custo, alto coeficiente de absorção no espectro de excitação do emissor de luz, alta fotoestabilidade (PLOTINO; GRANDE; MERCADE, 2019). Para aPDT ser eficaz, é essencial que o PS seja capaz de absorver a energia necessária com a fonte de luz utilizada (MEISEL; KOCHER, 2005). Em endodontia, diversas combinações de PS e fonte de luz foram testadas e obtiveram resultados divergentes (FEGAN; STEIMAN, 1995; JURIČ *et al.*, 2014; MORITZ *et al.*, 1997; ZAKARIASEN *et al.*, 1986; ZORITA-GARCÍA *et al.*, 2019). Cada um dos parâmetros da aPDT podem sofrer variações que são determinantes para o resultado da terapia. Concentração do PS, potência e tempo de irradiação, por exemplo, podem trazer eficácias diferentes a uma mesma combinação de PS e fonte de luz (NAGATA *et al.*, 2012).

Os PS mais estudados e empregados são o azul de metileno (MB), e o azul de toluidina (TBO). São moléculas anfifílicas, capazes de diluir em meios

hidrofóbicos e hidrofílicos. A absorção máxima para MB é de 660nm (BALL *et al.*, 1998; SEVERINO *et al.*, 2003), e a do TBO 630nm (USACHEVA; TEICHERT; BIEL, 2003). No que diz respeito a efeito antimicrobiano, MB e TBO tem capacidade de inativar bactérias gram positivas e gram negativas (NAGATA *et al.*, 2012), as quais compõem infecções endodônticas primarias (SIQUEIRA; RÔÇAS; LOPES, 2002).

Hoje em dia, as fontes de luz utilizadas se enquadram em três grupos: LASER, LED ou luz halógena (NAGATA *et al.*, 2012). Entre elas, os lasers de diodo, são mais fáceis de usar, mais baratos e portáteis, sendo os equipamentos mais utilizados para aPDT. Os lasers de baixa potência, tem comprimentos de onda que variam da luz visível azul ou vermelha, para os infravermelhos (PLOTINO; GRANDE; MERCADE, 2019).

O intervalo de tempo entre a inserção do PS e a aplicação da fonte de luz é chamada de pré-irradiação. Esse período é de suma importância porque permite que o PS penetre na dentina, no interior da célula bacteriana e exerça sua capacidade antimicrobiana (FIGUEIREDO *et al.*, 2014). Os tempos de pré-irradiação ainda não são um consenso na literatura. De acordo com Wainwright, o tempo de pré-irradiação mais curto trará danos apenas à membrana celular, enquanto tempos mais longos causarão danos também ao DNA da célula (WAINWRIGHT, 1998). Enquanto isso, Figueiredo sugere que tempos mais longos de pré-irradiação estejam relacionados a maior manchamento coronáro (FIGUEIREDO *et al.*, 2014).

Mesmo que aparentemente tenhamos diversas vantagens na utilização da aPDT, algumas limitações também são apresentadas na literatura endodôntica. Manchamento coronário pelo uso dos PS como o MB (CARVALHO *et al.*, 2011; FIGUEIREDO *et al.*, 2014; RAMALHO *et al.*, 2017). Porém, o uso desse corante em menores concentrações (0,01%) não causou manchamento dentário (GEORGE; KISHEN, 2007; ZANIN *et al.*, 2005). Também, quando foi utilizado em associação com o hipoclorito de sódio a 2,5% e o Endo-PTC (10% peroxido de ureia, 15% tween80 e 75% carbowax) não causou o manchamento, sendo possível removê-lo de forma completa da cavidade pulpar após o uso. Outra desvantagem é que o PS é uma substancia viscosa que acaba penetrando

a superfície dentinária, podendo formar uma lama dentinária, trazendo a obliteração dos túbulos dentinários. Dessa forma, uma irrigação final com o intuito de remover essa smear layer é desejável (SOUZA *et al.*, 2017).

A toxicidade dos PS é similar aos irrigantes endodônticos de uso comum (GOMES-FILHO *et al.*, 2016; XU *et al.*, 2009). Por conta disso, são recomendados cuidados clínicos similares aos sugeridos com os irrigantes (GAMBARINI *et al.*, 2011). Lasers com alta potência demandam maior cuidado quando utilizados em tecidos dentários e de suporte, tendo em vista que o aumento da temperatura na região pode induzir trauma aos tecidos de suporte e causar uma lesão irreversível. Diferentemente, a aPDT não causa nenhum efeito térmico no dente ou tecidos adjacentes (SOUKOS *et al.*, 2006).

Em ensaios laboratoriais a aPDT foi testada e obteve resultados promissores no auxílio a desinfecção após o CD (GURSOY *et al.*, 2013; NG *et al.*, 2011; SILVA *et al.*, 2012, (VENDRAMINI *et al.,* 2020). Ensaios clínicos avaliando a aPDT são poucos, com parâmetros técnicos como PS e fonte de luz variáveis e com resultados ainda controversos (AHANGARI *et al.*, 2017; ASNAASHARI *et al.*, 2017; GARCEZ *et al.*, 2007; MORSY *et al.*, 2018). Existem apenas duas revisões de literatura avaliando redução de carga microbiana no sistema de canais radiculares, por meio de ensaios clínicos. Uma não faz distinção entre dentes permanentes ou decíduos, entre infecção primária ou secundaria, e ainda entre tratamento endodôntico cirúrgico ou não cirúrgico (POURHAJIBAGHER; BAHADOR, 2019). A outra restringe para infecções secundárias, tratamentos endodônticos cirúrgicos e não cirúrgicos (NUNES *et al.,* 2022). Por esse motivo, um compilado da evidência científica a respeito do efeito da aPDT na redução da carga microbiana em tratamentos endodônticos não cirúrgicos de dentes permanentes com infecção primária ainda é necessário.

Sendo assim, a presente tese de doutorado objetivou avaliar a capacidade da aPDT em reduzir a carga microbiana no interior dos canais radiculares de dentes permanentes com infecção endodôntica primária quando usada após a desinfecção químico-mecânica convencional.

3 ARTIGO CIENTÍFICO

Title Does photodynamic therapy reduce bacterial load in teeth with primary endodontic infection? A Systematic Review and meta-analysis

Abstract

Introduction: The aim of this systematic review was to evaluate whether photodynamic therapy (aPDT) yields a greater microbial load reduction than chemo-mechanical root canal disinfection.

Methods: An electronic literature search was conducted on four major databases until august/2020 with no language or publication period restrictions. Clinical trials and randomized clinical trials in which participants presented primary endodontic infection in permanent teeth and which compared microbial load before and after using aPDT were included. Two researchers independently screened the title and abstract to define studies eligibility. Risk of bias and methodological quality of the studies were accessed. The review was registered in PROSPERO (CRD42020181783).

Results: Seven studies were included in qualitative analysis, and five were eligible for meta-analysis. In the fixed effect model, aPDT significantly enhanced root canal disinfection compared to standard protocols for cleaning and shaping ($p<0.05$). Subgroup analysis suggests that aPDT presents higher effect on decreasing anaerobes microorganisms' load.

Conclusions: The present study shows that the use of aPDT as an adjunct of chemo-mechanical disinfection is effective in promoting additional reduction of endodontic microbial load. The compilated data suggests that aPDT should help to improve the results of root canal treatments in permanent teeth with primary endodontic infection.

Keywords: Antimicrobial Photodynamic Therapy, Root Canal Disinfection, Endodontics

Introduction

In teeth presenting apical periodontitis, periapical healing directly depends on root canal disinfection (1). Root canal treatment (RCT) must focus in reducing microbial load to avoid that periapical disease development or maintenance be sustained (2). Several adjunct therapies have been suggested to optimize root canal disinfection of cleaning and shaping (3–5).

Antimicrobial photodynamic therapy (aPDT) is an adjunct protocol used to reduce bacterial load after chemo-mechanical root canal disinfection (CD) (6,7). It consists of using a photosensitizer (PS), i.e methylene blue and Toluidine blue, activated by a specific light source that promotes a phototoxic response (8–10). The reactive oxygen species that are created can cause damage to the bacterial cells leading to its death (8,9,11).

In endodontics, aPDT has been used with different light sources and photosensitizers (12–16). Preclinical data suggests that aPDTis a promising adjunctive supplement after CD (17,18). However, clinical studies evaluating microbial load reduction are scarce and present controversial results (19–21). Up to date, the only systematic review focused on the effect of aPDT did not distinguish primary and permanent teeth, primary and secondary infection and surgical or non-surgical endodontic treatment (22). Thus, the compilation of the available evidence regarding the effect of of aPDT in the reduction of microbial load in non-surgical endodontic treatment of permanent teeth with primary infection is still required.

This review was conducted to evaluate whether aPDT yields a greater microbial load reduction than CD. The research question framed for this systematic review was: Does aPDT used as supplementary disinfection protocol in primary endodontic treatment of teeth with chronic apical periodontitis provide a greater infection reduction than in treatments without aPDT?

Materials and methods

Registration and protocol

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement) (23). The review was registered with the international prospective register of systematic reviews (PROSPERO; Center for Reviews and Dissemination, University of York; and the National Institute for Health Research) under the registration number CRD42020181783. The clinical question was formulated and organized using the PICOS strategy: Population (P): permanent teeth with primary endodontic infection endodontically-treated, Intervention (I): antimicrobial photodynamic therapy (aPDT) after CD, Comparison (C): CD, Outcome (O): reduction of microbial load, Study design (S): clinical trials (CT) and randomized clinical trials (RCT).

Two independent reviewers (RMQ and LSP) conducted all systematic review stages. When necessary, two senior reviewers (PMPK, RKS) decided disagreements between the reviewers by consensus.

Eligibility Criteria

CT and RCT in which participants undergone root canal treatment of permanent tooth presenting primary endodontic infection and that compared root canal microbial load immediately after CD with that sampled after using aPDT were included. Manuscripts with unavailable full-text were excluded.

Information sources, search, study selection, data collection process

The electronic literature search on EMBASE (https://www.embase.com), PubMed (MEDLINE) (https://pubmed.ncbi.nlm.nih.gov/), Scopus (http://www.scopus.com) and Web of Science (https://www.webofknowledge.com) was conducted until august 10 (2020), with no language or publication period restriction. Gray literature was not included in searches because does not undergo peer review. The search terms used were a combination of the following: ‘endodontics OR tooth root canal OR dental canal’ AND ‘apdt OR antimicrobial photodynamic therapy OR antimicrobial photodynamic therapy OR photodynamic therapy' adapted for each database and followed their syntax rules (Table 1). After the identification in the databases, the

studies were imported into EndNote Web software® (https://www.myendnoteweb.com) to remove duplicates. Initially, articles were selected based on their title and abstract. After a full-text analysis, studies were included in the review if they met the eligibility criteria. A manual search was also conducted at the reference lists of the full-text selected studies to identify potentially relevant investigations that might have been missed during the initial search.

Table 1. Search strategy used and results for each electronic database (Embase, PubMed, Scopus, Web of Science).

Databases	Search	Query	Items found
Embase		('endodontics'/exp OR endodontics OR 'endodontics' OR 'tooth root canal'/exp OR 'canal, dental root' OR 'canal, tooth root' OR 'canalis radicis dentis' OR 'dental canal' OR 'dental root canal' OR 'root canal' OR 'root canal depth' OR 'root canal, tooth' OR 'root dental canal' OR 'tooth root canal' OR 'tooth periapical disease'/exp OR 'dental granuloma' OR 'dentin granuloma' OR 'dentine granuloma' OR 'periapical abscess' OR 'periapical disease' OR 'periapical diseases' OR 'periapical granuloma' OR 'periapical infection' OR 'periapical lesion' OR 'periapical periodontitis' OR 'tooth granuloma' OR 'tooth periapical disease' OR 'tooth periapical granuloma' OR 'tooth periapical infection' OR 'pulpitis'/exp OR pulpitis OR 'acute dental pulpitis' OR 'dental pulp inflammation' OR 'endodontal infections' OR 'endodontal inflammation' OR 'endodontic infection' OR 'endodontic inflammation' OR 'pulp infection' OR 'pulpal infection' OR 'pulpal inflammation' OR 'pulpitis' OR 'tooth pulp infection' OR 'tooth pulp inflammation' OR 'tooth pulpitis') AND (apdt OR 'antimicrobial photodynamic therapy'/exp OR 'antimicrobial photodynamic therapy' OR 'photodynamic therapy'/exp OR 'photodynamic therapy' OR 'photodynamic treatment' OR 'photosensitisation (intentional)' OR 'photosensitization (intentional)' OR 'therapeutic photosensitisation' OR 'therapeutic photosensitization' OR 'therapy, photodynamic' OR 'phototherapy'/exp OR phototherapy OR 'light therapy' OR 'phototherapy' OR 'laser therapy' OR 'laser disinfection' OR 'photoactivated disinfection')	565
PubMed		(endodontics OR root canal OR tooth root canal OR dental canal) AND (apdt OR antimicrobial photodynamic therapy OR phototherapy OR photoactivated disinfection OR photodynamic therapy OR light disinfection OR light therapy OR laser disinfection)	1404
Scopus		(endodontics OR root AND canal OR tooth AND root AND canal OR dental AND canal) AND (apdt OR antimicrobial AND photodynamic AND therapy OR phototherapy OR photoactivated AND disinfection OR photodynamic AND therapy OR light AND disinfection OR light AND therapy OR laser AND disinfection)	1084

Web of Science	ALL=(endodontics OR root canal OR tooth root canal OR dental canal) AND ALL=(apdt OR antimicrobial photodynamic therapy OR phototherapy OR photoactivated disinfection OR photodynamic therapy OR light disinfection OR light therapy OR laser disinfection)	572

Data extraction

Both reviewers tabulated microbiological load data (mean and standard deviation of the number of colony formation units – CFU – after CD and after aPDT) at an independent sheet in Microsoft Office Excel 2019 software® (Microsoft Corporation, Redmond, WA, USA). Intraclass Correlation Coefficient accessed interobserver agreement for microbial load data acquisition and showed perfect agreement (ICC=1). Information regarding authors, publication year, sample size, teeth anatomical/position characteristics, evaluated groups, moments of microbial sample acquisition (baseline, after CD, after aPDT), culture condition (aerobic or anaerobic), method of microbial load quantification, main outcome, and aPDT protocol (photosensitizer and its concentration, light source, fiber diameter, wavelength, energy, power, and irradiation time) was also collected.

Methodological quality and risk of bias assessment

The two reviewers, previously calibrated by discussing each checklist item, evaluated methodological quality and risk of bias of the included studies. Any disagreement was solved as described above. Methodological quality was evaluated using CONSORT statement checklist (24) to randomized clinical trials and TREND statement checklist (25) to non-randomized studies of interventions (NRSI) clinical trials. Risk of bias was evaluated using the Risk of Bias 2 tool (RoB2) (26) to randomized clinical trials and ROBINS-I tool (27) NRSI.

RoB2 analyses 5 domains: (1) randomization process; (2) deviations from intended interventions; (3) missing outcome data; (4) measurement of the outcome; (5) selection of the reported result. Also, it gives an overall risk of bias score: low risk of bias (LR); some concerns (SC); high risk of bias (HR). Selected studies were judged as having LR, SC, or HR in each entry using RevMan software, version 5.4 (The Cochrane Collaboration, Denmark). When there was

no risk of bias in the five categories mentioned above, the study was classified with a low risk of bias. When at least one of the domains was unclear, the study was regarded as having some concerns at risk of bias. When at least one of these criteria showed a high risk of bias, the study was considered as having a high risk of bias.

ROBINS-I have the first three domains that differs from RoB2 because the randomization process, if properly implemented protects against biases before the intervention. The final four domains are not protected by the randomization process, therefore remains the same used for RoB2 tool. ROBINS-I analyses 7 domains (1) bias due to confounding; (2) selection of participants into the study; (3) classification of interventions; (4) deviations from intended interventions; (5) missing data; (6) measurement of outcomes; (7) selection of the reported result.

Assessment of the quality of the evidence

The overall quality of the evidence used for estimating the effect of aPDT on the microbial load of teeth with chronic apical periodontitis was assessed by Grading of Recommendations Assessment, Development and Evaluation (GRADE). The assessment is made to the following domains: risk of bias, inconsistency, indirectness, imprecision, and other considerations (publication bias, large effect, plausible confounding, and dose response gradient). In the first 4 domains, quality of the evidence should be downgraded if “serious” or “very serious” risks were found; publication bias may be either suspected (downgraded) or undetected; large effect can be classified as no, large, or very large; plausible confounding would reduce demonstrated effect or would suggest spurious effect; and dose response gradient can be classified as yes or not. After the assessment, an overall rating of body evidence is rated as high, moderate, low and very low (28–30).

Synthesis of results

Data entry and synthesis of results were conducted in Microsoft Office Excel 2019 software®. In cases of missing data, authors were contacted via email. A second and third reminder were mailed to non-responders after 2 and 4

weeks. If authors did not respond, the respective studies were excluded from the meta-analyses.

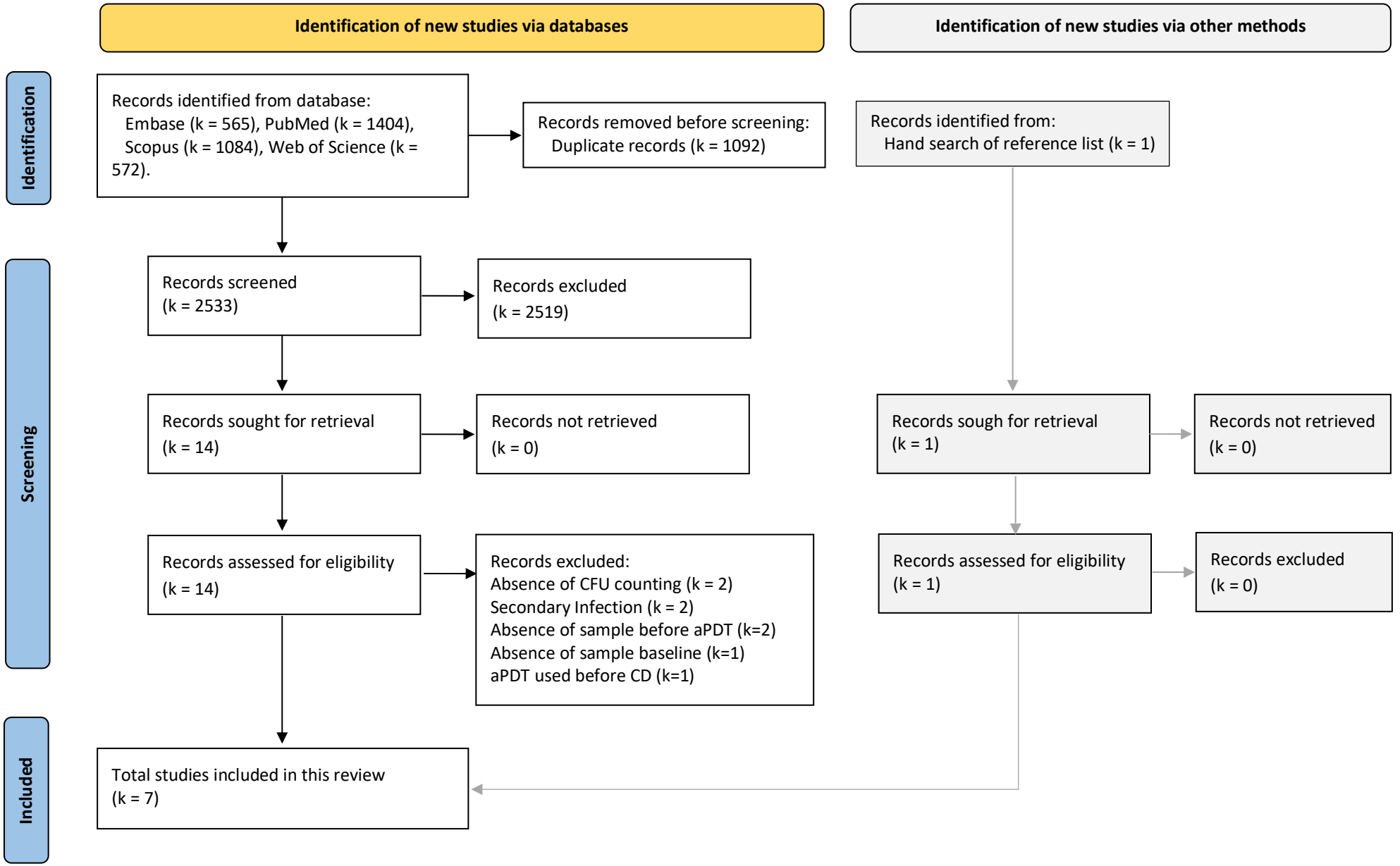
The effects protocols (CD or CD followed by aPDT) in reducing microbial load were calculated based on their differences in the number of CFU compared to the baseline (number of CFU before root canal CD). Therefore, higher values represent greater effect in root canal disinfection. Mean and standard deviation of after CD and aPDT effects were used for comparisons. In one study mean and standard deviation was missing (21), so imputation techniques were used to obtain estimates (31). Heterogeneity and publication bias was assessed by calculating the I^2 and the Egger test. Subgroup analyses were performed to determine if aPDT effect was influenced by the culture condition (aerobic and anaerobic). All calculations were conducted using the R software (www.r-project.org, version 4.1.0).

Results

Studies Selection

Initial screening in all databases revealed 3625 studies, of which 565 were found in Embase, 1404 in PubMed, 1084 in Scopus, and 572 in Web of Science. After discarding duplicates, 2533 papers were eligible for title reading, from which 86 were selected for abstract analysis. As 72 were excluded because did not fulfill the eligibility criteria, 14 studies remained for full-text assessment. Manual method was used by hand searching on reference list and one study was included (32). Finally, after the 15 full-text analysis, 7 articles were included in the qualitative synthesis (Figure 1). Two studies did not present CFU data as mean or median at each microbial load acquisition (33,34), being excluded from the meta-analyses. One of the five studies suitable for meta-analysis (21,32,35–37) showed independent data for each culture condition (36) (aerobic and anaerobic).

Figure 1. PRISMA flow diagram for studies retrieved through the searching and selection process.



Characteristics of the included studies

The main characteristics of the included studies are described in Tables 2 and 3. Publication dates ranged from 2008 (35) to 2019 (34,37). All studies, except one that does not mention tooth characteristics (34), included anterior or single rooted teeth. The sample size ranges from 15 (32) to 70 teeth (34).

Three clinical trials cultured samples at aerobic conditions (32,35,37), one at anaerobic environment (21) and three tested both microbial conditions (33,34,36). All studies used the number of CFU to evaluate microbial load reduction (Table 2).

Most of the included studies used Diode Laser as light source with wavelength between 660 to 980 (21,32,35,36). The other three investigations used LED light source (37), Nd:YAG laser (33) or Er:YAG laser (34). Three studies did not mention the photosensitizer used previous the laser application (33,34,36). One investigation employed methylene blue (21) and another Toluidine Blue (37). Polyethyleneimine and chlorine were used as photosensitizer in two studies (32,35) (Table 3).

Table 2. Summary of the included studies characteristics.

#	Authors/year	Sample size	Anatomical/position teeth characteristic	Evaluated groups	Microbial sample acquisition	Culture condition	Microbial load quantification	Main outcome
1	(#)Garcez et al. 2008a (35)	20	Anterior	• Chemo-mechanical disinfection + NaOCl 2,5% + EDTA 17% + aPDT + 1week $Ca(OH)_2$	First appointment: • After endodontic acess • **(#)** After CD • **(#)** After aPDT Second appointment: • After 1week with $Ca(OH)_2$ • After a new aPDT after 1week with $Ca(OH)_2$	Aerobic	• **(#)** CFU mean, SD for each sample • Log10 CFU Reduction mean, SD for each sample and tooth	The use of aPDT as an adjuvant to conventional endodontic therapy reduces the bacterial load, and a second aPDT is more effective than the first.
2	(#)Garcez et al.2008b(32)	15	Anterior	• Chemo-mechanical disinfection + aPDT + 1week $Ca(OH)_2$ paste • Conventional endodontic therapy +1week $Ca(OH)_2$ paste	First appointment: • After endodontic access • **(#)** After CD • **(#)** After aPDT Second appointment: • No microbial sample acquisition	Aerobic	• **(#)** CFU mean, reduction (%) for each sample	The use of PDT added to conventional endodontic treatment leads to a further major reduction of microbial load.
3	Lindstrom et al. 2017(33)	43	Single rooted	• Chemo-mechanical disinfection + NaOCL 1% • Chemo-mechanical disinfection + Saline + aPDT	First appointment: • fter endodontic access • After CD • After aPDT First apointment: • after two to four days without dressing	Anaerobic Aerobic	• CFU Scores divided into 5 categories (Heavy, Medium, Sparce, Very Sparce, None)	After intervention, both groups group yielded predictable negative bacterial samples.
4	(#)Morsy et al. 2018 (36)	56	Maxillary anterior	• Chemo-mechanical disinfection + aPDT simulation • Chemo-mechanical disinfection + aPDT	First appointment: • After endodontic access • **(#)** After CD • **(#)** After aPDT Second appointment: • After 1week without dressing	Aerobic Anaerobic	• **(#)** CFU/ml mean, SD for each sample in anaerobic and aerobic condition	The aPDT may be a successful adjunct to CD of necrotic cases with chronic periapical lesions in terms of postoperative pain and root canal disinfection.

					• After a new cleaning and shapping and a new aPDT 1week without dressing			
5	(#)Rabello et al. 2017 (21)	24	Single rooted	• Chemo-mechanical disinfection + 2 week $Ca(OH)_2$ paste + aPDT • Conventional endodontic therapy + aPDT	First appointment: • After endodontic access • **(#)** After CD • **(#)** After aPDT Second appointment: • After 2week with $Ca(OH)_2$ • After a new aPDT after 2 weeks with $Ca(OH)_2$	Anaerobic	• **(#)** CFU/ml median, range, median of reduction (%), number of positive cultures for each sample • Endotoxin contents (EU/ml) median, range, median of reduction (%), number of positive cultures for each sample	aPDT optimized the disinfection of bacteria from root canals in one-visit but not for two visit treatment with the accomplishment of calcium hydroxide medication. Regardless of the treatment modality, the supplemental aPDT was not effective against endotoxins.
6	Razumova et al. 2019 (34)	70	NI	• Chemo-mechanical disinfection + NaOCl 3% + PUI +EDTA 17% + aPDT • Chemo-mechanical disinfection + NaOCl 3% + PUI + EDTA 17%	• After endodontic access • After CD • After aPDT	NI	• CFU/ml for each MO in each sample.	The efficiency of root canal treatment with aPDT is quite high compared to CD without aPDT.
7	(#)Zorita-García et al. 2019 (37)	42	Single rooted	• Chemo-mechanical disinfection + NaOCl 5,25% + aPDT	• After endodontic access • **(#)** After CD # • **(#)** After aPDT #	Aerobic	• **(#)** CFU mean, SD, Reduction (%) and total CFU in each tooth sample	aPDT as an adjunct to CD can help to reduce the bacterial load.

Abbreviations: NI, not informed; NaOCl , sodium hipoclorite; EDTA, ethylenediaminetetraacetic acid; aPDT, antimicrobial photodynamic therapy; $Ca(OH)_2$, calcium hydroxide; CFU, colony forming units; PUI, passive ultrasonic irrigation; MO, microorganism; CD, chemo-mechanical root canal disinfection; **(#)** data used in metanalysis; SD, standard deviation.

Table 3. aPDT protocol of included studies.

#	Authors/year	PS/concentration (mg/ml)	Light source	Fiber diameter (μm)	Wavelength (nm)	Energy (J)	Power (mW)	Irradiation time (s)
1	Garcez et al. 2008a (35)	Polyethyleimine (PEI) Chlorin(e6[ce6])/-	Diode Laser (MMOptics)	300	660	9.6	40	240
2	Garcez et al.2008b(32)	Polyethylenimine and Chlorine(e6)/-	Diode Laser (MMOptics)	200	660	9.6	40	240
3	Lindstrom et al. 2017(33)	NI	Nd:YAG (Fotona Fidelis II+)	200	1064	NI	1500	NI
4	Morsy et al. 2018 (36)	NI	Diode Laser (Lite Medics)	200	980	NI	1200	20
5	Rabello et al. 2017(21)	Methylene Blue/ 0,1	Diode Laser (MMOptics)	300	660	NI	60	120
6	Razumova et al. 2019(34)	NI	Er:YAG	NI	2940	0.04	500-8400	NI
7	Zorita-García et al. 2019 (37)	Toluidine Blue/ 0,1	LED (Fotosan 630, CMS Dental)	500	630	NI	NI	60

Abbreviations: PS: Photosensitizer; NI: not informed. MMOptics (São Carlos, SP, Brazil); Fotona Fidelis II+ (Fotona DD, Ljubljana, Slovenia); Lite Medics (Milan, Italy); Fotosan 630 (CMS Dental, Glyngore, Denmark)

Results of the meta-analysis

Forest plot shows the results of meta-analysis (Figure 2). According to the fixed effect model aPDT significantly enhanced root canal disinfection with a SMD=0.38, i^2=71%. The random effect model also points in the same direction with higher disinfection for aPDT (SMD=0.35, i^2=71%), although not reaching statistical significance. In addition, subgroup analysis suggested that aPDT is more effective among anaerobes (SMD=0.74, i^2=80%) than aerobes (SMD=0.24, i^2=67%) microorganisms.

Despite a level of heterogeneity was observed (i^2=71%), assessment of publication bias by the Egger test found no indication of relevant asymmetry (p=0.799).

Methodological quality of the studies included

Four randomized clinical trials included (21,32,33,36) were evaluated by CONSORT statement checklist (Table 4). Only one study title did not identify the study design as a randomized clinical trial (32). Only Rabello and Morsy studies presented trial registration (item 23), but only Morsy's registry was available online (item 24). For this reason, items 3b and 6b were only accessed in this study and we did not found changes in methods or outcomes. The other 3 included studies were considered not applicable. Sample size determination was described in three studies (21,33,36). One did not present how sample size has been reached (32). Randomization items (8a, 8b, 9, 10), such as sequence generation, allocation concealment mechanism and implementation were not described only in one study (32). Blinding items (11a and 11b) were not described also in Garcez study. Result section items (13a to 19) were not described in Garcez study, while the remain authors have reported all items with exception of number 17a that one author (33) did not describe estimated effect size and its precision, and number 19 (harms) that the four studies did not show. Limitations (item 20) of the studies were found in three of them, only Garcez did not present any limitations on the text. Sources of funding were described by two authors (33,36).

Figure 2 - Forest plots of the meta-analysis of aPDT over CD. (A) Forest plot over both culture condition (aerobic and anaerobic); (B) Forest plot over aerobic condition; (C) Forest plot over anaerobic condition.

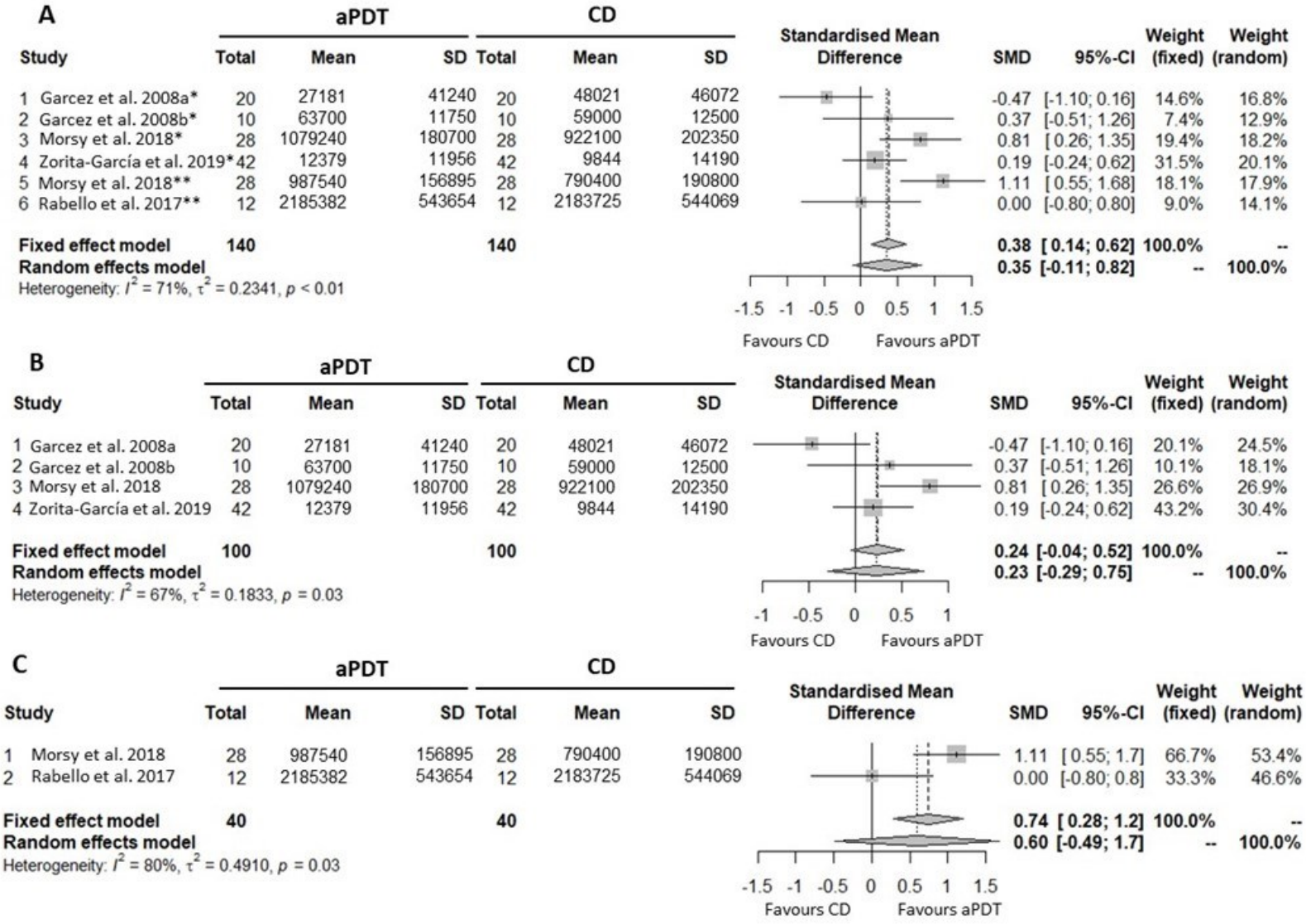


Table 4: CONSORT statement checklist.

Checklist itens	Garcez et al. 2008b	Lindstrom et al. 2017	Morsy et al. 2018	Rabello et al. 2017
1a	N	Y	Y	Y
1b	Y	Y	Y	Y
2a	Y	Y	Y	Y
2b	Y	Y	Y	Y
3a	Y	Y	Y	Y
3b	-	-	Y	-
4a	Y	Y	Y	Y
4b	Y	Y	Y	Y
5	Y	Y	Y	Y
6a	Y	Y	Y	Y
6b	-	-	Y	-
7a	N	Y	Y	Y
7b	-	-	-	-
8a	N	Y	Y	Y
8b	N	Y	Y	Y
9	N	Y	Y	Y
10	N	Y	Y	Y
11a	N	Y	Y	Y
11b	-	-	-	-
12a	Y	Y	Y	Y
12b	-	-	-	-
13a	N	Y	Y	Y
13b	N	Y	Y	Y
14a	N	Y	Y	Y
14b	-	-	-	-
15	N	Y	Y	Y
16	N	Y	Y	Y
17a	N	N	Y	Y
17b	-	-	-	-
18	-	-	-	-
19	N	N	N	N
20	N	Y	Y	Y
21	Y	Y	Y	Y
22	Y	Y	Y	Y
23	N	N	Y	Y
24	N	N	Y	N
25	N	Y	Y	N
Total	11	25	30	26
% Y	37,93%	86,20%	96,77%	89,65%
%N	62,07%	13,80%	3,23%	10,35%

*Abbreviations:*Y, yes; N, no.

The three NRSI included (34,35,37) were evaluated by TREND statement checklist (Table 5). For title and abstract items, all studies did not present information on how units were allocated to interventions but fulfill the other items. Background information and explanation of rationale were available in all three studies introductions. At methods section item 3 corresponds to participants, how and where they were recruited, Razumova study was the only one that did not describe all items (3a, 3b, 3c, 3d). Intervention protocol was evaluated in item 4 and describes what was given, how was the intervention protocol, where was it made and who carried out the intervention, and activities to increase adherence. All three studies did not describe item 4h, which refers to incentives to the participants. Razumova did not present item 4e, which corresponds to the

location of the intervention. Zorita-García did not describe item 4d, which corresponds to who carried out the intervention. Garcez describes all items between 4a to 4g. Only Zorita-Garcia has described hypothesis and specific objectives (item 5). Item 6a was presented by all studies and corresponds to clearly define outcome measures, item 6b refers to the methods used to collect and enhance quality of measurements, this last item was not described in Razumova study. Sample size (item 7) determination was not described in any study evaluated. Assignment method was only applicable in Razumova study, but only item 8a was described, which corresponds to the unit of assignment. Method used to assign and minimize potential bias such as stratification, blocking or matching was not reported. Blinding of any kind (participant, clinician or outcome assessment) was not presented by any authors. Statistical methods were well described in two studies (35,37), Razumova did not reported how statistical analyses were made. Participant flow items (12a to 12g) were the same to Garcez and Zorita-Garcia studies, both did not present flowchart information on enrollment and was not applicable to allocation, follow up and protocol deviations. Razumova did not present flowchart information on enrollment and in analysis, also was not applicable to follow up and protocol deviations. Only Zorita-garcía had described dates defining periods of recruitment (item 13). Baseline demographic and clinical characteristics of participants were not reported by any author (item 14a). Baseline characteristics specific conditions (item 14b) to study was described in Garcez and Zorita-García studies. Baseline equivalence was only possible in Razumova study, but it was not described (item 15). Item 17a, which corresponds to precision in measurements was not reported in only one study (34). At discussion section, Razumova did not discussed any items (20a to 20d) while Zorita-Garcia discussed all the items proposed. Garcez did not discuss only about the limitations or potential bias (20a).

Table 5: TREND statement checklist.

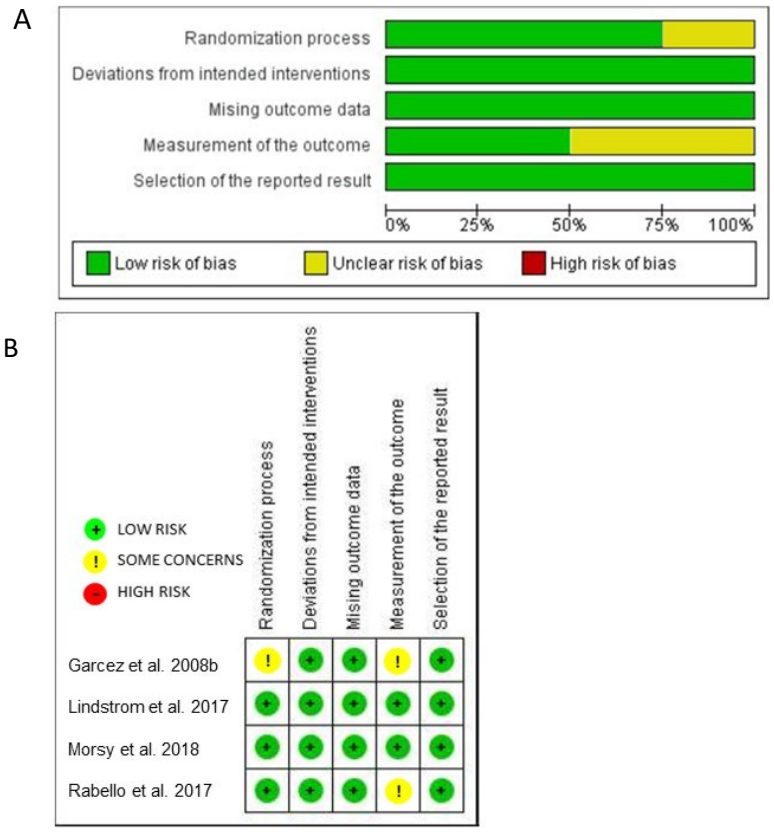
Checklist Itens	Garcez et al. 2008a	Razumova et al. 2019	Zorita-García et al. 2019
1a	N	N	N
1b	Y	Y	Y
1c	Y	Y	Y
2a	Y	Y	Y
2b	-	-	-
3a	Y	N	Y
3b	Y	N	Y
3c	Y	N	Y
3d	Y	N	Y
4a	Y	Y	Y
4b	Y	Y	Y
4c	Y	Y	Y
4d	Y	Y	N
4e	Y	N	Y
4f	Y	Y	Y
4g	Y	Y	Y
4h	N	N	N
5	N	N	Y
6a	Y	Y	Y
6b	Y	N	Y
6c	-	-	-
7	N	N	N
8a	-	Y	-
8b	-	N	-
8c	-	N	-
9	N	N	N
10a	Y	Y	Y
10b	-	-	-
11a	Y	N	Y
11b	Y	N	Y
11c	-	-	Y
11d	Y	N	Y
12a	N	N	N
12b	N	N	N
12c	Y	Y	Y
12d	-	Y	-
12e	-	-	-
12f	Y	N	Y
12g	-	-	-
13	N	N	Y
14a	N	N	N
14b	Y	N	Y
14c	-	-	-
14d	-	-	-
15	-	N	-
16a	Y	Y	Y
16b	-	-	-
17a	Y	N	Y
17b	-	-	-
17c	-	-	-
18	-	-	-
19	-	-	-
20a	N	N	Y
20b	Y	N	Y
20c	Y	N	Y
20d	Y	N	Y
21	Y	Y	Y
22	Y	Y	Y
Total	30	17	33
%Y	75%	37,70%	80,48%
%N	25%	62,30%	19,52%

*Abbreviations:*Y, yes; N, no.

Risk of Bias in the studies included

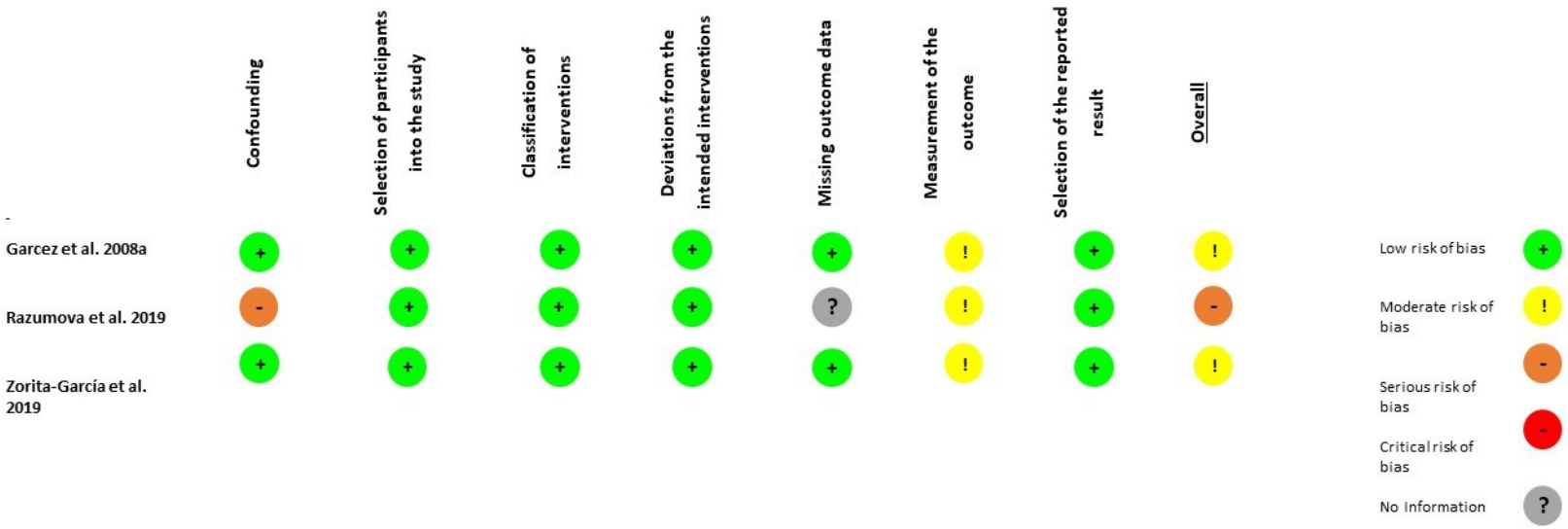
RoB2 showed no high risk of bias in all domains for all studies. Some concerns was found at the randomization process (32) and measurement of the outcome (21,32) domains. Two studies (21,32) were classified as some concerns and the others as low risk of bias (33,36) (Figure 3).

Figure 3 – Results of the risk of bias assessment using RoB2 to RCT studies. (A) Bar chart overview of RoB2 domains per study and overall risk of bias. (B) Traffic light plot of RoB2 included studies assessment.



ROBINS-I identified a serious risk of bias for one study (34) that showed one domain (confounding) with a serious risk of bias, one (missing outcome data) with no information and another (measurement of the outcome) with moderate risk. The other two investigations were classified as moderate risk of bias (34,35,37) as they had the measurement of the outcome domain with moderate risk of bias (Figure 4).

Figure 4 –Traffic light plot of ROBINS-I to NRSI included studies assessment.



Quality of the evidence

The quality of the available evidence for the effect of aPDT on the microbial load in teeth with chronic apical periodontitis was overall moderate. The reason for downgrading was imprecision because the included investigations presented wide confidence intervals and low sample sizes.

Discussion

The present study demonstrated that the use of aPDT after CD improve root canal disinfection. Both models (fixed and random) showed the beneficial effect of aPDT (figure 2), even though the random model did not reach classical statistical significance. This finding could be explained by the reduced number of published studies addressing this subject.

Findings of subgroup analysis revealed positive effect of aPDT as a supplementary disinfection protocol in both aerobic and anaerobic species, which encourage its use in mixed infections and could be beneficial for persistent infections. It is known that in tooth with persistent apical periodontitis the microbial flora is characterized by one or just a few species, predominantly Gram-positive microorganisms with an equal distribution of facultative and obligate anaerobes (38,39). Different factors related to these microorganisms, including the ability to form biofilms, invading dentinal tubules, and to survive in nutritional starvation, contribute to its extremely challenging elimination from the root canal system during cleaning and shape (39,40). As demonstrate herein, the use of aPDT after CD improve root canal disinfection, which might contribute for successful treatment outcome.

To the best of the authors knowledge, this is the first review on the effect of aPDT in the reduction of microbial load in non-cirurgical endodontic treatment of permanent teeth with primary infection. The only systematic review on this topic did not distinguish neither primary from secondary endodontic infections nor permanent from deciduous teeth (22). As demonstrate herein, the authors found a significant effect of aPDT in reducing bacterial load; however, the heterogeneity found among the studies was very high (I_2=94.18%), reducing the evidence quality. In the present study, restrictions were made seeking better comparisons

in meta-analysis. This explains the reduced number of studies included in this research.

The included studies in this systematic review concluded that aPDT used as a supplementary protocol has similar or better capacity to reduce bacterial load from the root canal than CD. All of them used single rooted tooth, which is related to a lower anatomical complexity. However, Gutknecht et al. (2004) reported that 980nm diode laser can help disinfection deep into the dentin (41). These findings associated with the ones reported in this review, suggest that aPDT could also be effective to disinfect complex areas such as isthmuses and lateral canals which are more frequently found in posterior teeth. Future clinical trials aiming to investigate the efficacy of aPDT after CD to reduce bacterial load in teeth with complex anatomy are necessary.

Other supplementary disinfection protocols have been studied and compared to CD followed by aPDT. CD plus calcium hydroxide dressing before the aPDT protocol showed that the beneficial effect of the photodynamic therapy was washed out when calcium hydroxide dressing was used (21). Calcium hydroxide is a well-known interappointment dressing that achieves a high pH value by releasing highly reactive hydroxyl ions, which affects bacterial cytoplasmic membranes, proteins and DNA (42,43). Calcium hydroxide is also the only intracanal dressing clinically effective against endotoxin, which plays an important role in maintenance of periapical lesions and is related to clinical symptoms (44,45,43,46). In this regard, considering literature findings, the use of aPDT after CD could reach a canal disinfection similar to that provided by the calcium hydroxide dressing without the need for a second visit for root canal filling.

Regarding the effect of aPDT on endotoxin, only one included investigation addressed this topic (21). In this study the aPDT was performed using red diode laser (660 nm wavelength,60 mW power, 129J/cm2 energy density) and methylene blue as photosensitizer, and it was not effective against endotoxin. A recent in vitro study demonstrated that the photosensitizer directly influences the aPDT effectiveness to reduce endotoxin from the infected root canal system (47). Considering this hypothesis, new clinical trials, modifying the photosensitizer and the aPDT protocol are necessary to understand its real effect on the endotoxin.

Considering that persistent infection in the root canal system could be the reason of endodontic failure, reduce the microbial load inside this system is an important goal during endodontic treatments (1,2,48,49). To understand the effect of aPDT on this topic, some authors investigated the apical repair after endodontic treatments of teeth with apical periodontitis using an animal model (50,51). However, clinical trials evaluating the effectiveness of aPDT on the apical repair are still lack. Aiming to reach the best evidence quality, the present review focused on understand the effect of aPDT on the reduction of the root canal system bacterial load in clinical trials. Future clinical investigations on the periapical repair after endodontic treatments of teeth with apical periodontitis using different disinfection protocols, including aPDT, are needed. Well conducted clinical trials on this topic will contribute with the understanding of the impact of reducing the microbial load inside the root canals on the clinical outcome: endodontic success.

This review included specific methodological stages to increase transparency and strength: the review protocol was registered *a priori*; four major databases were used to collect data; the methodological quality and RoB of the included studies was assessed using the CONSORT, TREND, ROBINS-I and RoB2 tools; and the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA Statement) was used to report this study (23). PRISMA statement is intended to facilitate transparent, complete and accurate reporting of systematic reviews, what should benefit users of reviews, such as health care providers.

Differences in the aPDT protocols between the included studies represents a limitation of this review. Although aPDT have been used in endodontics for a long time, there is still a lack of guidelines over the parameters that should be set clinically. Therefore, clinical trials included in this review had heterogeneous parameters (table 3). Moreover, two studies (34,32) included only 37% of the CONSORT and TREND checklist items, being considered studies of low methodological quality. Regarding the risk of bias, investigations with moderate (35,37) and unclear (21,32) risk may have led to weaker evidence. It is important to point out that only one study showed serious risk of bias (34) and it was not suitable for the meta-analysis.

The resulting evidence was graded as moderate as included investigations showed imprecision. In this regard, the current evidence regarding the effect of aPDT on the microbial load of teeth with chronic apical periodontitis need to be confirmed by future clinical studies. Experimental designs with large sample size and standardize protocols are needed, resulting in a high level of evidence for clinical decision making.

Conclusions

Despite the limitations of the available evidence, the present study shows that aPDT plays an important role in endodontic treatments of primary infected teeth, reducing the microbial load of the root canal system. Moreover, the current data suggests that future clinical trials should be designed based on specific guidelines to standardize research procedures, increasing strength, and avoiding bias.

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4 CONSIDERAÇÕES FINAIS

A instrumentação mecânica e irrigação com substâncias químicas auxiliares, empregadas no tratamento endodôntico convencional (CD) tem-se mostrado insuficiente para debelar a infecção presente no sistema de canais radiculares (LIN *et al.*, 1991; SIQUEIRA *et al.*, 1997; VERA *et al.*, 2012). Sendo assim, o presente estudo revisou a literatura acerca da capacidade da aPDT reduzir a carga microbiana no interior dos canais radiculares de dentes permanentes com infecção endodôntica primária quando empregada após o CD. Com este objetivo, foram incluídos estudos clínicos que compararam a carga microbiana do canal radicular imediatamente após a CD com a carga microbiana do canal radicular após o CD e aPDT. A meta-análise evidenciou uma menor carga microbiana quando a aPDT foi empregada como protocolo de desinfecção suplementar tanto em espécies aeróbicas como em anaeróbicas. Tal achado incentiva o uso da aPDT em infecções mistas, podendo ser benéfica para casos de infecções persistentes.

Em dentes com periodontite apical persistente a flora microbiana é caracterizada por uma ou poucas espécies, predominantemente microrganismos Gram-positivos com uma distribuição igual de anaeróbios facultativos e obrigatórios (SAKAMOTO *et al.*, 2009; SIQUEIRA; RÔÇAS, 2021). Diferentes fatores relacionados a esses microrganismos, incluindo a capacidade de formar biofilmes, invadir os túbulos dentinários e sobreviver na inanição nutricional, contribuem para sua eliminação extremamente desafiadora do sistema de canais radiculares (NEELAKANTAN *et al.*, 2017; SIQUEIRA; RÔÇAS, 2021). Como demonstrado aqui, o uso de aPDT após CD melhora a desinfecção do canal radicular, o que pode contribuir para o sucesso do tratamento endodôntico.

Outros protocolos complementares de desinfecção foram estudados e comparados ao CD seguido de aPDT. CD mais curativo de hidróxido de cálcio antes do protocolo aPDT mostrou que o efeito benéfico da terapia fotodinâmica foi eliminado quando o curativo de hidróxido de cálcio foi usado (RABELLO *et al.*, 2017). A literatura mostra que a pasta de hidróxido de cálcio é capaz e alterar

as membranas citoplasmáticas bacterianas, proteínas e DNA (ESTRELA *et al.*, 1994; MOHAMMADI; DUMMER, 2011) e é eficaz contra a endotoxina, que desempenha importante papel na manutenção das lesões periapicais e está relacionado aos sintomas clínicos (LEONARDO *et al.*, 2004; MARTINHO *et al.*, 2017; MOHAMMADI; DUMMER, 2011; TANOMARU *et al.*, 2003). Nesse sentido, considerando os presentes achados, o uso de aPDT após CD poderia alcançar uma desinfecção do canal semelhante à proporcionada pelo curativo de hidróxido de cálcio sem a necessidade de uma segunda visita para obturação do canal radicular.

Contudo, a literatura a cerca desta temática ainda apresenta algumas limitações. Todos os estudos incluídos nesta revisão empregaram dentes uniradiculares, o que está relacionado à menor complexidade anatômica. Em um estudo in vitro, Gutknecht et al. (2004) relataram que o laser de diodo de 980nm pode ajudar na desinfecção profunda da dentina (GUTKNECHT *et al.*, 2004). Para confirmar se a aPDT é eficaz para reduzir a carga microbiana em dentes que apresentam áreas mais complexas, como istmos e canais laterais, mais frequentemente encontrados em dentes posteriores, futuros ensaios clínicos são necessários.

Além disso, o efeito da aPDT na endotoxina bacteriana foi avaliado em apenas uma investigação (RABELLO *et al.*, 2017), apesar de não ter sido demonstrado um efeito benéfico da aPDT em relação a endotoxina, um recente estudo in vitro demonstrou que o fotossensibilizador influencia diretamente a eficácia da aPDT para reduzir a endotoxina do sistema de canais radiculares infectados (COUTO *et al.*, 2021). Considerando essa hipótese, novos ensaios clínicos, modificando o fotossensibilizador e o protocolo aPDT são necessários para entender seu real efeito sobre a endotoxina.

Da mesma forma, ainda faltam ensaios clínicos avaliando a eficácia da aPDT no reparo apical. Considerando que a infecção persistente no sistema de canais radiculares pode ser a causa do insucesso endodôntico, reduzir a carga microbiana dentro deste sistema é uma meta importante durante os tratamentos endodônticos (NAIR, 2006; SIQUEIRA, 2001; SIQUEIRA; RÔÇAS, 2008; SJÖGREN *et al.*, 1997). Para entender o efeito da aPDT neste tópico, apenas

modelos animais foram empregados (BORSATTO *et al.*, 2016; SILVA *et al.*, 2019). Ensaios clínicos bem conduzidos neste tópico contribuirão para a compreensão do impacto da redução da carga microbiana no interior dos canais radiculares no resultado clínico: sucesso endodôntico.

Considerando os achados da presente investigação, pode-se afirmar que as evidências disponíveis sobre o efeito da aPDT na carga microbiana de dentes com periodontite apical crônica são escassas. Desta forma, os resultados aqui demonstrados precisam ser confirmados por estudos clínicos futuros com desenhos experimentais que incluam um grande tamanho amostral e protocolos de emprego da aPDT padronizados. Tais investigações contribuirão com um alto nível de evidência para a tomada de decisão clínica.

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