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Instituto de Ciência e Tecnologia

BEATRIZ KOWALSKI FIAMINI

AVALIAÇÃO CLÍNICA DOS EFEITOS ANTIMICROBIANOS E ANTI-INFLAMATÓRIOS DE MEDICAÇÕES INTRACANAL ASSOCIADAS A N-ACETIL CISTEÍNA

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Área: Endodontia. Linha de pesquisa: patogenia, imaginologia e terapias em odontologia.

Orientadora: Profa. Dra. Marcia Carneiro Valera

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IMPACTO POTENCIAL DESTA PESQUISA

O tratamento endodôntico busca a máxima redução de microrganismos no sistema de canais radiculares em níveis compatíveis com a cura na região periapical. Para alcançar esse objetivo, são utilizadas diversas estratégias de desinfecção, como o preparo biomecânico, associado à irrigação abundante com substâncias químicas auxiliares, seguido pela aplicação de medicação intracanal, o que irá potencializar a descontaminação durante o tratamento (Ørstavik, 1996; Siqueira; Rôças, 2008). Além disso, a medicação intracanal pode contribuir para melhorar a resposta inflamatória periapical do hospedeiro (Ørstavik, 1996; Siqueira; Rôças, 2008).

O $Ca(OH)_2$ é uma medicação intracanal bem estabelecida em Endodontia, e atua no combate dos microrganismos e seus produtos, e na modulação da inflamação periapical (Estrela; Holland, 2003; Siqueira; Lopes, 1999). No entanto, sua ação é limitada, uma vez que a difusão de íons Ca^{++} e OH^- na dentina é dificultada por sua capacidade tampão (Nerwich; Figdor; Messer, 1993; Wang; Humfa, 1988), além disso, essa difusão varia ao longo do comprimento do canal radicular, sendo o terço cervical mais eficaz em relação ao terço apical (Nerwich; Figdor; Messer, 1993). Como resultado, as bactérias dentro dos túbulos dentinários estão expostas ao $Ca(OH)_2$ apenas na entrada do canal (Sjögren et al., 1991). Além disso, pesquisas destacam que microrganismos multirresistentes podem sobreviver à medicação intracanal com $Ca(OH)_2$ (Guerreiro-Tanomaru et al., 2013; Pinheiro et al., 2003; Prada et al., 2019).

Neste contexto, a N-acetilcisteína (NAC) é uma substância química que possui ação antioxidante, anti-inflamatória, que atua contra patógenos e biofilme, sendo muito utilizada na área médica (Pérez-Giraldo et al., 1997).

Recentemente, nosso grupo de pesquisa realizou um estudo *in vivo* utilizando a NAC como medicação intracanal (Corazza et al., 2021), que demonstrou resultados satisfatórios no perfil inflamatório periapical, porém, notou-se que sua ação antimicrobiana não foi tão satisfatória, sugerindo que esse efeito poderia ser aumentado por meio da associação da NAC a outros antimicrobianos, visando, assim, o seu uso na clínica endodôntica. Na sequência, realizamos outro estudo *in vivo* (Martinho et al., 2022) utilizando a NAC e avaliando sua ação sobre patógenos endodônticos, a qual obteve resultados promissores (publicações referentes ao auxílio pesquisa FAPESP 2018/01703-9). Paralelamente, realizamos um estudo *in vitro* associando a NAC ao $Ca(OH)_2$ e obtivemos resultados promissores com total eliminação de *Enterococcus faecalis* (dados não publicados).

Portanto, neste estudo clínico randomizado, foi analisado *in vivo* as medicações intracanais: $Ca(OH)_2$ + soro fisiológico; $Ca(OH)_2$ + NAC e NAC + CHX, a fim de avaliar a ação combinada da NAC com antimicrobianos comumente utilizados na Endodontia sobre microrganismos e seus produtos, e os efeitos dessas medicações sobre o processo inflamatório na região periapical. Essa abordagem visou oferecer novas perspectivas antimicrobianas e anti-inflamatórias.

POTENTIAL IMPACT OF THIS RESEARCH

Endodontic treatment aims for maximal reduction of microorganisms in the root canal system at levels compatible with healing in the periapical region. To achieve this, various disinfection strategies are employed, such as biomechanical preparation, coupled with abundant irrigation with auxiliary chemical substances, followed by intracanal medication application, enhancing decontamination during treatment (Ørstavik, 1996; Siqueira; Rôças, 2008). Additionally, intracanal medication may contribute to improving the periapical inflammatory response (Ørstavik, 1996; Siqueira; Rôças, 2008).

Calcium hydroxide ($Ca(OH)_2$) is a well-established intracanal medication in Endodontics, combating microorganisms and their products, and modulating periapical inflammation (Estrela; Holland, 2003; Siqueira; Lopes, 1999). However, its action is limited, as the diffusion of Ca^{++} and OH^- ions in dentin is hindered by its buffering capacity (Nerwich; Figdor; Messer, 1993; Wang; Humfa, 1988), further varying along the root canal length, with the cervical third being more effective than the apical third (Nerwich; Figdor; Messer, 1993). Consequently, bacteria within dentinal tubules are exposed to $Ca(OH)_2$ only at the canal entrance (Sjögren et al., 1991). Moreover, research highlights that multidrug-resistant microorganisms may survive Minimum Inhibitory Concentration (MIC) with $Ca(OH)_2$ (Guerreiro-Tanomaru et al., 2013; Pinheiro et al., 2003; Prada et al., 2019).

In this context, N-acetylcysteine (NAC) is a chemical substance with antioxidant, anti- inflammatory properties, acting against pathogens and biofilms, widely used in the medical field (Pérez-Giraldo et al., 1997). Recently, our research group conducted an *in vivo* study using NAC as intracanal medication (Corazza et al., 2021), showing satisfactory results in periapical inflammatory profile. However, its antimicrobial action was not as effective, suggesting that combining NAC with other antimicrobials could enhance its use in endodontic clinics. Subsequently, another *in vivo* study (Martinho et al., 2022) assessed NAC's action on endodontic pathogens, yielding promising results (publications related to FAPESP research grant 2018/01703-9). Simultaneously, an *in vitro* study combining NAC with $Ca(OH)_2$ demonstrated promising results, with complete elimination of *Enterococcus faecalis* (unpublished data).

Thus, this randomized clinical study analyzed intracanal medications in vivo: $Ca(OH)_2$ + saline solution; $Ca(OH)_2$ + NAC, and NAC + CHX, aiming to evaluate the combined action of NAC with commonly used antimicrobials in Endodontics on microorganisms and their products, and the effects of these medications on the inflammatory process in the periapical region. This approach aimed to provide new antimicrobial and anti-inflammatory perspectives.

BANCA EXAMINADORA

Marcia Carneiro Valera Garakis (Orientador)
Universidade Estadual Paulista (Unesp)
Instituto de Ciência e Tecnologia
Campus São José dos Campos

Elaine Faga Iglecias
Universidade Paulista
Endodontia
Campus Bacelar

Claudio Antonio Talge Carvalho
Universidade Estadual Paulista (Unesp)
Instituto de Ciência e Tecnologia
Campus São José dos Campos

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RESUMO

Fiamini BK. Avaliação clínica dos efeitos antimicrobianos e anti-inflamatórios de medicações intracanal associadas a N-acetil Cisteína [dissertação]. São José dos Campos (SP): Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia; 2024.

A interação entre fatores microbianos em canais radiculares com polpa necrótica e a reação inflamatória contra a invasão desses agentes agressores nos tecidos perirradiculares do hospedeiro ocasiona o desenvolvimento, progressão e disseminação da periodontite apical. Este estudo clínico randomizado teve como objetivo avaliar a desinfecção dos canais radiculares e o processo inflamatório periapical após o preparo biomecânico e o uso de diferentes medicações intracanais (MIC), monitorando o perfil bacteriano; níveis de endotoxinas; modulação inflamatória através da quantificação de citocinas inflamatórias em dentes com infecções endodônticas primárias e periodontite apical. Foram relacionados os níveis de endotoxinas e perfil microbiano com sinais e sintomas clínicos; e a quantidade de citocinas foi correlacionada com o volume da lesão periapical (mm^3), através do uso de tomografia computadorizada de feixe cônico (TCFC). Para atingir o objetivo proposto, 29 dentes permanentes e unirradiculares apresentando necrose pulpar e lesão periapical foram submetidos à terapia endodôntica em sessão múltipla. Foram coletadas amostras do interior dos canais após a abertura coronária (S1); após o preparo biomecânico (S2), e após 14 dias da MIC (S3), com hidróxido de cálcio + solução salina fisiológica ($Ca(OH)_2$ + soro fisiológico); hidróxido de cálcio + N-acetil cisteína ($Ca(OH)_2$ + NAC); e N-acetil cisteína + clorexidina (NAC + CHX). Os conteúdos coletados foram analisados quanto ao perfil microbiológico por *Checkerboard DNA-DNA hybridization* e quantificação de endotoxinas pelo teste *Limulus Amebocyte Lysate - LAL*. Também foram feitas coletas do fluido intersticial apical após o preparo biomecânico (SF1) e após 14 dias de MIC (SF2) para a quantificação de citocinas inflamatórias (IL-1β, IL-6, IL-17 e TNF-α) pelo ensaio multiplex. Todos os dados obtidos foram tabulados no software *GraphPad Prism* 8 e foram analisados estatisticamente. O teste de Dunn comparou espécies bacterianas entre grupos de MIC, enquanto o teste de Kruskal-Wallis comparou níveis de LPS. A correlação entre o volume das lesões periapicais e as citocinas inflamatórias foi analisada pelo teste de Spearman ($p < 0,1$). Em relação às espécies bacterianas, LPS e sinais e sintomas clínicos, a NAC combinada com $Ca(OH)_2$ ou CHX mostrou resultados semelhantes ao grupo controle ($Ca(OH)_2$ + solução salina). As citocinas inflamatórias IL-1β e TNF-α demonstraram correlação com o volume de destruição óssea periapical em casos de infecção endodôntica primária em dentes unirradiculares, sugerindo que ambas podem influenciar diretamente o processo de reabsorção óssea periapical. Conclui-se que diferentes MICs associadas a NAC são eficazes na desinfecção dos canais radiculares, equiparando-se ao $Ca(OH)_2$ + soro fisiológico, que já é uma MIC bem estabelecida em Endodontia, e a IL-1β e o TNF-α se correlacionaram positivamente com a destruição óssea periapical.

Palavras-chave: Acetilcisteína; Citocinas; Clorexidina; Endotoxinas; Hidróxido de Cálcio; Periodontite apical.

ABSTRACT

Fiamini BK. Clinical evaluation of antimicrobial and anti-inflammatory effects of intracanal medications associated with N-acetyl cysteine [dissertation]. São José dos Campos (SP): São Paulo State University (Unesp), Institute of Science and Technology; 2024.

The interaction between microbial factors in root canals with necrotic pulp and the inflammatory response against these aggressive agents in the host's periradicular tissues leads to the development, progression, and dissemination of apical periodontitis. This randomized clinical trial aimed to evaluate root canal disinfection and the periapical inflammatory process following biomechanical preparation and the use of different intracanal medications (MIC). The study monitored the bacterial profile, endotoxin levels, and inflammatory modulation through the quantification of inflammatory cytokines in teeth with primary endodontic infections and apical periodontitis. Endotoxin levels and microbial profiles were correlated with clinical signs and symptoms, while cytokine levels were correlated with the volume of the periapical lesion (mm^3) using cone-beam computed tomography (CBCT). To achieve the proposed objective, 45 permanent, single-rooted teeth with pulp necrosis and periapical lesions underwent multi-session endodontic therapy. Samples were collected from the canal interiors after coronal opening (S1), after biomechanical preparation (S2), and after 14 days of MIC treatment (S3) using calcium hydroxide + saline solution ($Ca(OH)_2$ + saline solution); calcium hydroxide + N-acetylcysteine ($Ca(OH)_2$ + NAC); and N-acetylcysteine + chlorhexidine (NAC + CHX). The collected contents were analyzed for microbial profile using Checkerboard DNA-DNA hybridization and for endotoxin quantification using the Limulus Amebocyte Lysate (LAL) test. Apical interstitial fluid samples were also collected after biomechanical preparation (SF1) and after 14 days of MIC (SF2) for the quantification of inflammatory cytokines (IL-1β, IL-6, IL-17, and TNF-α) using a multiplex assay. All data obtained were tabulated in GraphPad Prism 8 software and statistically analyzed. Dunn's test compared bacterial species between MIC groups, while the Kruskal-Wallis test compared LPS levels. The correlation between periapical lesion volume and inflammatory cytokines was analyzed using Spearman's test ($p < 0.1$). Regarding bacterial species, LPS, and clinical signs and symptoms, NAC combined with $Ca(OH)_2$ or CHX showed results similar to the control group ($Ca(OH)_2$ + saline solution). The inflammatory cytokines IL-1β and TNF-α showed a correlation with the volume of periapical bone destruction in cases of primary endodontic infection in single-rooted teeth, suggesting that both may directly influence the process of periapical bone resorption. It is concluded that different MICs combined with NAC are effective in disinfecting root canals, comparable to $Ca(OH)_2$ + saline solution, which is already a well-established MIC in Endodontics, and that IL-1β and TNF-α positively correlate with periapical bone destruction.

Keywords: Cytokines; Chlorhexidine; Endotoxins; Calcium hydroxide; N-acetyl cysteine; Apical periodontitis.

SUMÁRIO

1 INTRODUÇÃO

A polpa dental é um tecido conjuntivo frouxo altamente especializado contido no interior da cavidade pulpar que desempenha funções muito importantes para a homeostasia do complexo dentino-pular, dentre elas, a formação de dentina, resposta sensorial e resposta imunológica (Demarco et al., 2011; Tennert et al., 2014). Quando a polpa é sujeita a estímulos agressores, como trauma dental ou exposição à cárie profunda, a escassez de circulação colateral diminui o aporte sanguíneo nesta região, e logo, o tecido pulpar torna-se suscetível à lesão irreversível, que pode culminar com sua rápida degeneração e posterior necrose tecidual (Tennert et al., 2014).

O caráter microbiano das infecções endodônticas primárias é representado por bactérias Gram-negativas e Gram-positivas (Gomes et al., 2004; Rôças, Siqueira e Santos, 2004), que estão envolvidas com os sintomas clínicos e com a patogênese das reações inflamatórias periapicais (Assed et al., 1996; Sundqvist, 1992). As bactérias Gram-negativas possuem lipopolissacarídeos (LPS) na membrana externa da parede celular, que são endotoxinas e potentes agentes inflamatórios liberados durante a morte e reprodução celular (Pitts, Williams e Morton, 1982). O LPS é um padrão molecular associado a patógenos (PAMP) que, ao ser reconhecido por seus receptores de reconhecimento padrão (PRRs) em células de resposta imune, como os macrófagos, inicia a resposta do hospedeiro para eliminar o agente agressor. Frente à liberação do LPS, o reconhecimento dessa substância pelo sistema imunológico ocorre através dos receptores *Toll-like*, que também são responsáveis por identificar ligantes endógenos induzidos durante a resposta inflamatória (Akira, Takeda e Kaisho, 2001).

A resposta inflamatória do hospedeiro às infecções endodônticas é influenciada por citocinas e quimiocinas inflamatórias, fundamentais para a sinalização celular e com um papel crucial na modulação das respostas imunológicas diante da infecção endodôntica, essas proteínas desempenham funções vitais, incluindo ativação, proliferação, diferenciação, sobrevivência e apoptose dos linfócitos (Burska; Boissinot; Ponchel, 2014; Seymour; Taylor, 2000). Elas são secretadas por diferentes tipos celulares presentes nos tecidos lesados,

desempenham um papel fundamental na modulação da atividade celular durante o processo inflamatório, alterando as funções celulares ou recrutando células de defesa para a região agredida (Borish; Steinke, 2003). Além disso, as citocinas são subdivididas em citocinas que aprimoram as respostas imunológicas celulares (pró-inflamatórias) e aquelas que favorecem as respostas de anticorpos (anti-inflamatórias) (Burska; Boissinot; Ponchel, 2014). Em conjunto com as prostaglandinas, as citocinas, incluindo interleucinas e o fator de necrose tumoral-α (TNF-α), desempenham um papel fundamental na promoção e controle da resposta inflamatória, essas substâncias contribuem ativando e diferenciando os osteoclastos, células encarregadas da reabsorção óssea na região apical (Nair et al., 1996).

A interleucina 1β (IL-1β), produzida por diversos tipos celulares, incluindo macrófagos, osteoclastos, PMNs e fibroblastos, é um mediador eficaz da reabsorção óssea, tanto na osteoporose quanto em lesões perirradiculares em Endodontia (Fouad, 1997; He et al., 2020; Lim et al., 1994; Morsani et al., 2011). Estudos indicam que a IL-1β está correlacionada com sinais e sintomas clínicos, bem como com a extensão da destruição óssea periapical (Lim et al., 1994; Tani-Ishii; Wang; Stashenko, 1995). A interleucina 6 (IL-6), por sua vez, é crucial na remodelação óssea e na ativação e diferenciação de células do sistema imunológico e osteoclastos. Em lesões periapicais, a IL-6 está presente em níveis avançados e associada a casos sintomáticos, desempenhando um papel pró-inflamatório e contribuindo para a reabsorção óssea (Azuma et al., 2021; Jakovljevic et al., 2015).

O TNF-α é outro mediador inflamatório eficaz que induz a perda de massa óssea e é reconhecido como o principal mediador da resposta inflamatória aguda, especialmente quando desencadeada por bactérias Gram-negativas e outros micro-organismos infecciosos (Birkedal-Hansen, 1993). O TNF-α atua nas células estromais, aumentando a expressão de fatores de reabsorção óssea e promovendo a diferenciação dos osteoclastos, mesmo na ausência de níveis elevados de RANKL, exercendo um impacto significativo na diferenciação dos osteoclastos (Gupta, 2002; Lam, 2000).

Já a interleucina 17 (IL-17), inicialmente identificada como pró-inflamatória e secretada por células T ativadas, desempenha um papel crucial na defesa contra infecções bacterianas e fúngicas, mas quando em excesso, contribui para a inflamação crônica e persistente (Beringer; Noack; Miossec, 2016; McGeachy; Cua;

Gaffen, 2019; Moseley et al., 2003; Veerdonk et al., 2011). A ação benéfica ou prejudicial da IL-17 depende da quantidade produzida e da forma como seus sinais são recebidos e transmitidos dentro das células respondentes (McGeachy, Cua, Gaffen, 2019).

Assim, o tratamento de dentes com necrose pulpar e presença de lesão periapical, tem como objetivos a eliminação dos micro-organismos e seus produtos bem como prevenir a reinfecção intrarradicular, a fim de possibilitar condições favoráveis para a cura dos tecidos periapicais (Siqueira; Rôças, 2008). Para isso, é realizado o preparo biomecânico associado a substâncias químicas auxiliares como o hipoclorito de sódio, que dentre suas atividades, possui capacidade de dissolução de matéria orgânica e ação antimicrobiana (Haapasalo et al., 2010). No entanto, para complementar a antissepsia do sistema de canais radiculares e modular a inflamação na região apical e periapical, o uso de medicação intracanal (MIC) tem sido amplamente recomendado (Barbosa-Ribeiro et al., 2018).

O hidróxido de cálcio ($Ca(OH)_2$) tem sido utilizado como MIC por apresentar ação sobre micro-organismos, cuja atividade está relacionada à dissociação iônica em íons Ca2+ e OH-, além de possuir ação sobre LPS de bactérias Gram-negativas (Mohammadi; Dummer, 2011) e atuar no processo inflamatório periapical (Martinho et al., 2015). Entretanto, diversos estudos (Guerreiro-Tanomaru et al., 2013; Lima et al., 2015; Pinheiro et al., 2003, 2015; Prada et al., 2019) têm demonstrado que determinados micro-organismos podem resistir à ação do $Ca(OH)_2$ e das demais manobras de descontaminação adotadas durante o tratamento endodôntico (Guerreiro-Tanomaru et al., 2013; Lima et al., 2015; Mayer, 2015; Pinheiro et al., 2003; Prada et al., 2019). Assim, a associação do hidróxido de cálcio com outras substâncias, como a clorexidina (CHX), tem o potencial de ser utilizada como MIC de forma mais eficaz nas infecções endodônticas (Mohammadi; Dummer, 2011; Valera et al., 2010).

O uso da CHX associada ao hidróxido de cálcio aumenta a atividade antimicrobiana da medicação (Mohammadi; Abbott, 2009) uma vez que soma a ação da duas medicações (Signoretti et al., 2011) resultando em amplo espectro de ação antimicrobiana, ação antifúngica, propriedade da substantividade, cuja atividade no canal dura até 12 semanas e portanto seu uso pode minimizar a recontaminação, além de ser biocompatível (Mohammadi, Abbott, 2009). Entretanto, os trabalhos da

literatura mostram que ainda existe a necessidade da busca por uma medicação com maior atividade antimicrobiana, especialmente nos biofilmes bacterianos (Rabin et al., 2015) e que atue na resolução do processo inflamatório periapical.

Nesse contexto, a N-acetil cisteína (NAC) é uma substância química derivada da cisteína com ação antioxidante pela presença do grupo tiol (Samuni et al., 2013) que estimula a síntese de glutationa (GSH), atua na desintoxicação e eliminam espécies reativas de oxigênio (ROS) (Cacciatore et al., 2010). A NAC também tem capacidade de inibir as citocinas TNF-α, IL-1β e IL-6 (Palacio; Markert; Martínez, 2011) e se mostrou eficaz contra patógenos e biofilme endodônticos em estudos *in vitro* (Choi et al., 2018; Moon et al., 2016).

Em estudos clínicos realizados por nosso grupo de pesquisa, verificou-se que $Ca(OH)_2$ + CHX gel 2% e NAC mostraram uma atividade antimicrobiana mais ampla do que $Ca(OH)_2$ + soro fisiológico contra patógenos endodônticos na infecção primária do canal radicular (Martinho et al., 2022). Sendo assim, associar a NAC com outras substâncias químicas pode favorecer o aumento do espectro de ação antimicrobiano dessa substância. Além disso, a atividade antibiofilme associada a capacidade anti-inflamatória da NAC também demonstra grande potencial, uma vez que, além de combater a infecção, a terapia endodôntica terá melhores resultados quando se aumenta os eventos regenerativos intrínsecos dos tecidos perirradiculares danificados (Virdee et al., 2022).

A associação de NAC com CHX já foi testada *in vitro* apresentando resultados favoráveis como MIC contra biofilme de *Enterococcus faecalis* demonstrando a ação sinérgica dessa associação (Palaniswamy et al., 2016). Em estudo realizado *in vitro* por nosso grupo de pesquisa (dados ainda não publicados), também verificou-se excelente ação antimicrobiana da NAC associada ao $Ca(OH)_2$ sobre biofilme de *E. faecalis*. Além disso, a NAC interfere no processo de neoformação óssea em implantes, pois reduz a expressão do Ligante do receptor ativador do fator nuclear kappa B (RANKL) com consequente melhor osteointegração (Lee et al., 2013).

A diminuição da densidade mineral óssea causada pelos produtos bacterianos decorrentes da infecção endodôntica é visualizado na radiografia como uma área radiolúcida em torno do ápice radicular (Bender, 1997), e o tamanho da radiolucência periapical antes e após o tratamento endodôntico é rotineiramente

comparado com o uso de radiografia periapical (RP) em duas dimensões (2D). No entanto, de acordo com as recomendações da AAE (American Association of Endodontists) e AAOMR (American Academy of Oral and Maxillofacial Radiology) (EUA, 2015) a utilização da tomografia computadorizada de feixe cônico (TCFC) se faz necessária para análise de fatores pré-operatórios, tais como a presença e o tamanho real da lesão periapical, que desempenham um papel importante no resultado e no acompanhamento do tratamento endodôntico. Sendo assim, no presente estudo foi utilizada a TCFC para avaliar o volume das lesões periapicais.

Devido à busca contígua por medicamentos que atuem tanto na microbiota e produtos dos microrganismos presentes no sistema de canais radiculares, bem como que atuam no processo inflamatório periapical, e associada à ampla experiência de nosso grupo com estudos clínicos randomizados, a finalidade do presente projeto foi avaliar novas associações de medicações intracanal sobre microrganismos presentes no sistema de canais radiculares e seu papel no processo inflamatório periapical.

2 ARTIGOS

2.1 Artigo 1 – Fiamini, BK; Santos, AC; Guerrero, GG, Gagliardi, CF; Barbosa, CGC; Valera, MC. Perfil Microbiano e Ação sobre Subprodutos Bacterianos em Dentes com Infecção Endodôntica Submetidos ao Tratamento com Medicações Intracanais com N-Acetilcisteína: Estudo Clínico Randomizado / *Microbial Profile and Action on Bacterial Byproducts in Teeth with Endodontic Infection Treated with Intracanal Medications with N-Acetylcysteine: A Randomized Clinical Study*

RESUMO

N-acetilcisteína (NAC) apresenta uma alternativa promissora como medicação intracanal (MIC), dada sua capacidade de superar as limitações do hidróxido de cálcio ($Ca(OH)_2$). Este estudo clínico randomizado monitorou o perfil microbiano intracanal antes e após tratamento endodôntico com diferentes MIC e avaliou o efeito sobre endotoxinas de diferentes MIC combinadas com NAC em dentes com infecções endodônticas primárias e periodontite apical. Vinte e nove dentes de pacientes com infecções endodônticas primárias foram selecionados e divididos aleatoriamente em três grupos de acordo com a MIC: grupo hidróxido de cálcio: $Ca(OH)_2$ + solução salina fisiológica; grupo hidróxido de cálcio + N-acetil cisteína: $Ca(OH)_2$ + NAC; e grupo N-acetil cisteína + clorexidina gel 2%: NAC + CHX. Foram avaliados sinais e sintomas clínicos, e a coleta de amostras foi realizada em três momentos distintos: antes do preparo biomecânico (S1), após o preparo biomecânico (S2) e após a remoção da MIC (S3). Quarenta espécies bacterianas foram investigadas usando o método de *checkerboard DNA-DNA hybridization*, enquanto a quantificação de endotoxinas foi realizada usando o método cinético cromogênico ensaio cinético cromogênico de lisado de amebócitos de Limulus (QCL-LAL). Em relação às espécies bacterianas, LPS e sintomas clínicos, a NAC combinada com $Ca(OH)_2$ ou CHX mostrou resultados semelhantes ao grupo controle ($Ca(OH)_2$ + solução salina), que já é uma MIC bem estabelecida na Endodontia. Esses achados sugerem que a NAC representa uma abordagem promissora para o tratamento endodôntico, potencialmente oferecendo uma alternativa eficaz e segura para o tratamento de infecções endodônticas.

Palavras-chave: Acetilcisteína; Clorexidina; Endodontia; Endotoxinas; Hidróxido de Cálcio.

ABSTRACT

N-acetylcysteine (NAC) presents a promising alternative as an intracanal medication (MIC) due to its ability to overcome the limitations of calcium hydroxide ($Ca(OH)_2$). This randomized clinical study monitored the intracanal microbial profile before and after endodontic treatment with different MICs and evaluated the effect on endotoxins of different MICs combined with NAC in teeth with primary endodontic infections and apical periodontitis. Twenty-nine teeth from patients with primary endodontic infections were selected and randomly divided into three groups according to the MIC: calcium hydroxide group: $Ca(OH)_2$ + saline solution; calcium hydroxide + N-acetylcysteine group: $Ca(OH)_2$ + NAC; and N-acetylcysteine + 2% chlorhexidine gel group: NAC + CHX. Clinical signs and symptoms were evaluated, and sample collection was performed at three different time points: before biomechanical preparation (S1), after biomechanical preparation (S2), and after MIC removal (S3). Forty bacterial species were investigated using the checkerboard DNA-DNA hybridization method, while endotoxin quantification was performed using the chromogenic kinetic assay with Limulus amebocyte lysate (QCL-LAL). Regarding bacterial species, LPS, and clinical symptoms, NAC combined with $Ca(OH)_2$ or CHX showed similar results to the control group ($Ca(OH)_2$ + saline solution), which is already a well-established MIC in Endodontics. These findings suggest that NAC represents a promising approach for endodontic treatment, potentially offering an effective and safe alternative for the treatment of endodontic infections.

Keywords: Acetylcysteine, Calcium Hydroxide; Chlorhexidine; Endodontics, Endotoxins.

In primary endodontic infections, the pulp chamber provides a selective habitat for a mixed polymicrobial community comprising both Gram-negative and Gram-positive bacteria, predominantly anaerobic (1–4), where specific species have a direct correlation with the manifestation of clinical signs and symptoms (1).

Bacterial products, such as endotoxins, are fundamental in assessing the interactions between microorganisms and dental tissues, especially concerning the etiology and management of endodontic infections. Lipopolysaccharides (LPS) are virulence factors that compose mostly the outermost membrane of the cell wall of Gram-negative bacteria and play a significant role in perpetuating endodontic disease (5–7).

Endodontic treatment aims to reduce microorganisms and endotoxins in the root canals to levels that support effective healing. To achieve this objective, the use of intracanal medication (MIC) between the treatment sessions allows the reduction to levels compatible with healing (8,9), and the neutralization of the cytotoxic effects of LPS (5,10).

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Calcium hydroxide ($Ca(OH)_2$) has been used as an MIC in Endodontics for nearly a century (11), due to its biocompatibility (12) and its effectiveness in eradicating bacteria in root canals due to its alkaline properties and antimicrobial effects (11,13). Nevertheless, there are limitations to the use of $Ca(OH)_2$ in Endodontics. The diffusion of OH- ions in dentin is hindered by its buffering capacity (14,15), moreover, this diffusion varies along the length of the root canal, whereas, the cervical third shows greater diffusion effectiveness compared to the apical third (14). As a result, the bacteria within the dentinal tubules are only exposed to $Ca(OH)_2$ at the entrance of the canal (16). Furthermore, research emphasizes (17–19) that multidrug-resistant microorganisms may survive intracanal medication with $Ca(OH)_2$.

Chlorhexidine (CHX) is a non-phenolic biocide that serves as a chemical agent capable of inactivating a wide range of microorganisms (20), it also interacts with phospholipids and lipopolysaccharides (LPS) in the bacterial cell membrane (25). The association of CHX with $Ca(OH)_2$ presents a promising strategy to enhance the effectiveness of intracanal medication in treating endodontic infections (12,21). This synergy provides a broad spectrum of antimicrobial activity, including antifungal properties and the ability to remain active in the canal for an extended period, lasting up to 12 weeks (22,23).

However, despite substantial progress, scientific literature indicates an ongoing need to develop medications with even greater antimicrobial activity, particularly against bacterial biofilms (24). In this context, N-acetylcysteine (NAC) has been increasingly recognized in Endodontics, although it is not an antibiotic (25), recent studies have highlighted NAC for its antibacterial activity (25–34), particularly its ability to disrupt bacterial communities in biofilms (25,27,32). NAC is a chemical compound derived from cysteine that exhibits antioxidant properties due to the presence of a thiol group (35,36). This group stimulates the synthesis of glutathione (GSH) and plays a role in detoxification and the removal of reactive oxygen species (ROS) (37).

NAC's activity against endodontic pathogens has been tested in vitro against monospecies biofilms of Enterococcus faecalis (26,31–33), demonstrating its efficacy against planktonic bacteria (32,33) and biofilm formations (26,31,32). Additionally, in other in vitro studies where its activity was tested against multispecies biofilms (27–29), NAC also proved to be effective. NAC was combined with CHX (28,31), yielding

even more favorable results, indicating a potential synergy between these substances.

Therefore, this randomized clinical trial aimed to assess the effect of MICs combined with NAC on the disinfection and reduction of microbial load and their byproducts in root canals of teeth with primary endodontic infections and apical periodontitis. Additionally, the study evaluated the signs and symptoms of patients before and after the use of these medications.

Materials and Methods

This study was submitted to the Research Ethics Committee (REC) of the Institute of Science and Technology of São José dos Campos (ICT-SJC) – UNESP and received approval under the opinion number 5.828.002. It will subsequently be registered with the Brazilian Clinical Trials Registry (REBEC).

Patient Selection

All patients voluntarily participated in this study, having signed an informed consent form. A total of twenty-nine teeth were selected for the study based on specific inclusion and exclusion criteria. Enrollment occurred between June 2023 and April 2024. The selection focused on teeth that had a single root and exhibited periapical lesions due to primary endodontic infections, thus, these teeth had not undergone prior endodontic treatment. To ensure that the teeth met these requirements, thorough assessments were carried out using clinical examination, preoperative periapical radiography, and cone-beam computed tomography (CBCT) scans.

The study excluded teeth that had been previously treated endodontically, teeth that showed signs of periodontal disease or root fracture, and, patients who had used antifungal or antibiotic medications within the three months leading up to the endodontic treatment. Technical factors such as the inability to achieve proper rubber dam isolation or insert a paper point to the working length of the root canal were also grounds for exclusion.

Division of Experimental Groups

The teeth were divided into three groups:

a) *Group 1 - $Ca(OH)_2$ + saline solution - n=11;*
b) *Group 2 - $Ca(OH)_2$ + NAC - n=8;*
c) *Group 3 - NAC + CHX - n=10.*

To allocate participants into groups, randomization was conducted according to the CONSORT 2010 guidelines (38). This process involved drawing lots for the treatment type to be administered before the procedure.

Clinical Procedures and Sample Collection

Before the clinical intervention, all instruments used in this study underwent gamma sterilization with cobalt-60 by Ipen (Institute of Energy and Nuclear Research, São Paulo, SP, Brazil) to ensure complete elimination of pre-existing endotoxins. All subsequent steps were carried out aseptically.

Patients were first subjected to anesthesia and the teeth were isolated with a rubber dam. The tooth crowns were rinsed with physiological saline solution (Laboratório Sanobiol, Pouso Alegre, MG, Brazil), and the interface between the crown and the rubber dam was sealed using a photopolymerizable resin barrier (Top Dam – FGM Produtos Odontológicos, Joinville, SC, Brazil). Subsequently, prophylaxis of the tooth crown was performed using prophylactic paste (Vigodent S/A Indústria e Comércio, Rio de Janeiro, RJ, Brazil) and a Robinson brush (Microdont – Micro Usinagem de Precisão Ltda, São Paulo, SP, Brazil). The operative field was disinfected with sterile swabs (Jiangsu Medical Materials Co. Ltda, Jiangsu, China) moistened with 30-volume hydrogen peroxide (Byofórmula, São José dos Campos, SP, Brazil), followed by 5.25% sodium hypochlorite (NaOCl) (Byofórmula, São José dos Campos, SP, Brazil). To prevent chemical contamination of the root canal and interference with microbiological cultures, the NaOCl was then neutralized with 5% sodium thiosulfate (Byofórmula, São José dos Campos, SP, Brazil).

Access to the pulp chamber was achieved with manual irrigation of sterile saline solution. Upon completion of the coronal opening and confirmation of access to the pulp cavity, an initial root canal sample of the canal contents was collected

(S1).

For the S1 sample, 4 sterile paper points (Dentsply Maillefer Ind. E Com. Ltda, Petrópolis, RJ, Brazil) were inserted one at a time into the root canal to the predetermined WL (measured by CBCT and subtracted by 3 mm) and left in place for 60 seconds each. The first paper point introduced into the root canal was stored in a sterile, pyrogen-free Eppendorf tube (2 mL) containing 1 mL of pyrogen-free water (Equiplex Indústria Farmacêutica, Aparecida de Goiânia, GO, Brazil) for subsequent endotoxin analysis. Three paper points were stored in TE solution (10 mM Tris-HCl, 1 mM EDTA pH 7.6) for analysis using the checkerboard DNA-DNA hybridization method.

Biomechanical preparation (BMP) was carried out using the crown-down technique with the Reciproc® reciprocating system (VDW, Munich, Germany). Files were chosen based on the diameter of the K-file that best fit the root canal initially. When K-file #20 fit the root canal, the R40 file was used, while R50 file was used when K-file #30 fit the canal. WL was measured using the RomiApex apex locator (RomiBras LTDA, Rio de Janeiro, RJ, Brazil) and confirmed radiographically. The established WL was 1 mm short of the apical foramen.

Instrumentation was divided into thirds from crown to apex (cervical, middle, and apical), with the root canals being irrigated with 2.5% NaOCl solution (8 mL per third, for a total of 24 mL). After biomechanical preparation, the canals were filled with 17% EDTA and for 3 minutes, subsequently, the irrigating solution was agitated using three cycles of 20 seconds with the Irrisonic 20/01 ultrasonic tip (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) positioned 2 mm from the WL. The irrigation solution was refreshed between each agitation cycle with EDTA, totaling 1 mL.

Afterward, the root canal was flooded with 2.5% NaOCl and agitated using three cycles of 20 seconds with the Irrisonic 20/01 ultrasonic tip at 2 mm from the WL. The irrigation solution was refreshed between each cycle of NaOCl agitation, totaling 5 mL.

To neutralize the NaOCl used during the final irrigation protocol, the canal was irrigated with 5 mL of 5% sodium thiosulfate, followed by an irrigation with 10 mL of sterile, pyrogen-free saline solution.

The second samples were collected post-biomechanical preparation (S2) for

future microbiological and endotoxin analysis. The same methodology described previously was followed, with absorbent paper points inserted throughout the entire length of the root canal. Upon the second sample collection, patients were randomly assigned to one of three groups according to the MIC.

For Group 1, a paste of $Ca(OH)_2$ and saline was prepared in a 1:1 volume ratio. For the NAC + CHX group, the paste was also prepared in a 1:1 volume ratio. For Group 2 specifically, the association of NAC with $Ca(OH)_2$ was stabilized. To achieve this, 25 mg/mL of NAC (29,31,33) was diluted in saline solution, resulting in a pH of approximately 1,50. To increase the pH of the mixture, a 6 mol/L sodium hydroxide buffer solution was added until the pH reached 12. Finally, 1 g of $Ca(OH)_2$ was added to the resultant solution.

The medications were introduced into the root canal using pyrogen-free lentulo spirals until the canal was completely filled. Subsequently, the teeth were sealed with a layer of coltosol (Vigodent, Rio de Janeiro, RJ, Brazil), followed by temporary restoration using composite resin cement (Z100 3M ESPE, Sumaré, SP, Brazil).

After a 14-day interval following the first session, the patient returned to the clinic for the completion of the procedures. Anesthesia, isolation, and antisepsis of the operative field were conducted as described in the initial session. Upon accessing the pulp chamber, the MIC was removed using a sterile, pyrogen-free R40 file at the working length and a K file #30 at the length of the tooth to clean the apical foramen, along with irrigation using 20 mL of pyrogen-free saline solution. Subsequent irrigation of the root canal was performed with 8 mL of 2,5% NaOCl, followed by the final irrigation protocol, adhering to the parameters outlined in the initial session. After neutralizing the NaOCl from the previous step with 5% sodium thiosulfate and irrigating with 10 mL of sterile, pyrogen-free saline solution, the third sample collection (S3) was conducted in the same way as the previously described collections.

In cases with no pain, odor, and dry canals proceeded to the obturation stage using gutta-percha cones (Dentsply Maillefer Ind. Com. Ltda., Petrópolis, RJ, Brazil) and AH Plus root canal sealer (Dentsply DeTrey GmbH, Konstanz, Germany). A coronal seal with a layer of Coltosol (Vigodent, Rio de Janeiro, RJ, Brazil) followed by temporary restoration with composite resin cement (Z100 3M ESPE, Sumaré, SP,

Brazil) was then performed. A final radiograph was taken to verify adequate filling length and lateral sealing of the root canal.

Checkerboard DNA-DNA hybridization

The detection and semi-quantification of 40 bacterial species, as well as their levels and proportions, were performed using the checkerboard DNA-DNA hybridization method outlined by Socransky et al. (2004) (39). To each Eppendorf plastic tube containing samples and TE buffer solution, 100 µL of 0.5 M NaOH (Labsynth) was added so that the bacterial DNA remained viable for an extended period of time. The samples were then stored at -20 °C until further analysis.

DNA probes were prepared using the DIG DNA Labeling Kit (Roche Diagnostics, Indianapolis, IN) and frozen until use. The samples were heated for 10 minutes, and 800 µL of 5 mol/L ammonium acetate was added to promote bacterial lysis and subsequent suspension of DNA in solution. A positively charged nylon membrane (15 × 15 cm; Amersham Biosciences, Chicago, IL) was placed in a MiniSlot-30 apparatus (Immunetics, Cambridge, MA), and 1,000 µL of each suspension was placed into the extended slots and attached to the membrane by baking at 120 °C for 20 minutes. Each membrane contained 28 samples, with the last two channels reserved for controls composed of a mixture of microbial species at two concentrations (105 and 106 bacterial cells) for detection by DNA probes.

Hybridization of digoxigenin-labeled whole-genomic DNA probes, which run perpendicular to the channels of the clinical samples, was carried out using a Miniblotter-45 apparatus (Immunetics, Cambridge, MA). The bound probes were identified with phosphatase-conjugated antibodies to digoxigenin and chemiluminescence (CDP-Star Detection Reagent; GE Healthcare Limited, Little Chalfont, UK). Membranes were exposed to radiographic film (AGFA-IBF; Duque de Caxias, Rio de Janeiro, Brazil) for about 60 minutes. The films were analyzed, and each probe produced a specific signal that was compared visually with the signals from the 10^5 and 10^6 bacterial cell controls. The resulting signals were categorized into six different classes based on bacterial counts: 0, not detected; 1, $<10^5$ cells; 2, approximately 10^5 cells; 3, between 10^5 and 10^6 cells; 4, approximately 10^6 cells; and 5, more than 10^6 cells.

Quantification of Endotoxins

To evaluate the neutralization of endotoxins, samples collected from root canals were analyzed using the kinetic chromogenic Limulus Amebocyte Lysate (QCL-LAL) assay (Lonza, Walkersville, MD, USA). Initially, a standard curve was constructed using Escherichia coli endotoxin control, which was reconstituted with a specified volume of pyrogen-free water as indicated in the quality certificate. This suspension contained 50 EU/mL and was further diluted from this endotoxin standard to different concentrations (50; 5; 0,5; 0,05; 0,005 EU/mL). For each sample, a positive control (root canal content sample spiked with a known amount of endotoxin) was performed.

In a 96-well pyrogen-free plate, 100 µL of pyrogen-free water (reaction blank), endotoxin standards, root canal samples, and positive controls were added. The plate was incubated in the QCL kinetic reader at 37 ± 1 °C for 10 minutes. The reader was connected to a microcomputer with WinkQCL™ software for assay management, execution, and report generation. Subsequently, 100 µL of the kinetic chromogenic LAL reagent was added to each well of the plate using a multichannel pipette with pyrogen-free tips.

After starting the kinetic assay, the microplate reader software continuously monitored absorbance at 405 nm in each well. WinkQCL™ software automatically calculated a linear log-log correlation between the reaction time of each standard and the corresponding endotoxin concentration. The standard curve parameters were printed for analysis.

Signs and symptoms

Before the procedure, subjective examination gathered information on the current condition of the tooth and whether spontaneous pain was present or absent.

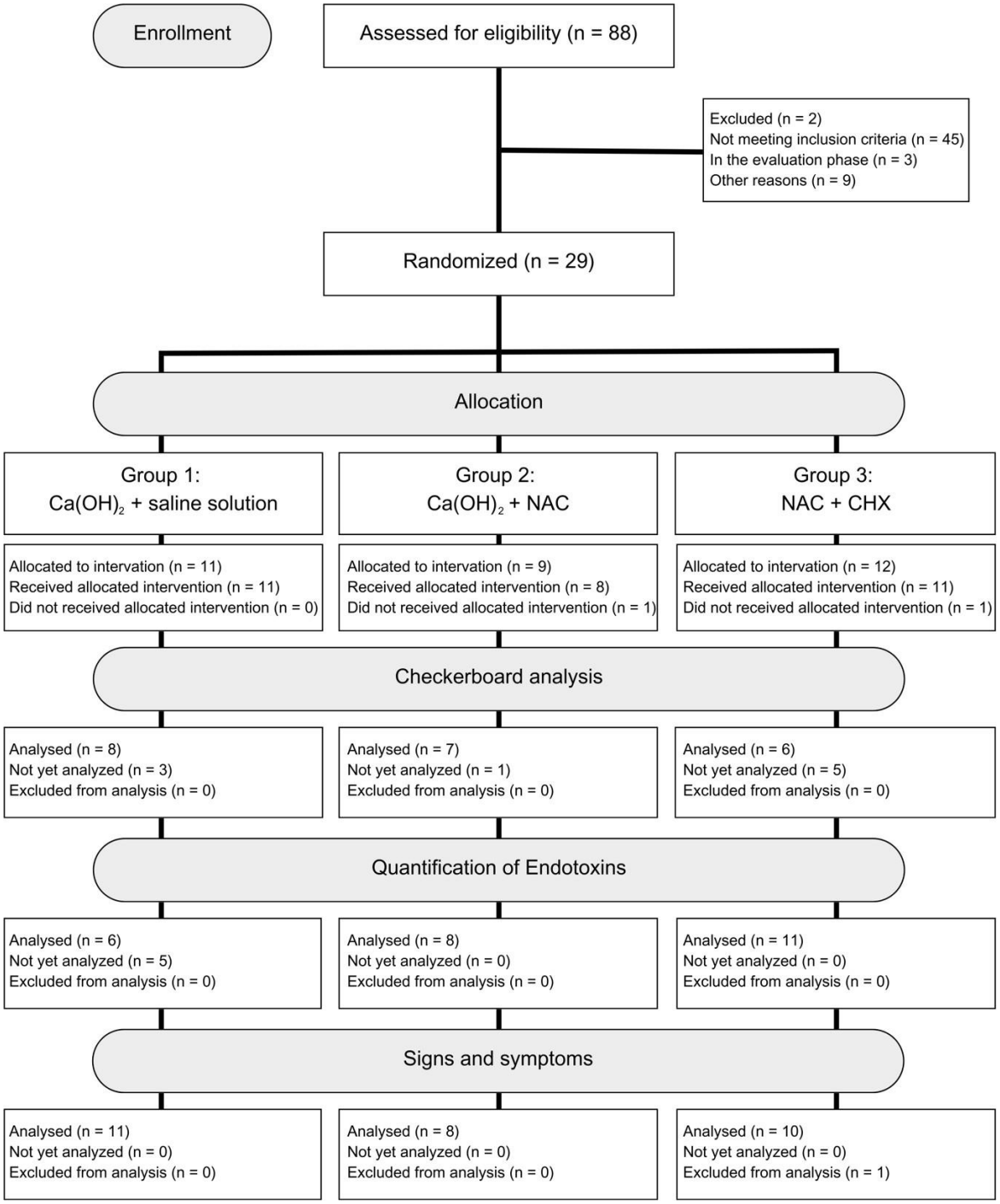
Objective examination included assessing swelling and fistulas, all of which were noted. Additionally, tests were conducted to assess sensitivity to percussion and palpation. All data were tabulated for subsequent analysis. At the end of the session, patients were provided with a visual analog scale adapted with Wong-Baker faces (VAS) for pain. The VAS scale, adapted with Wong-Baker faces (0–10) to aid

patient comprehension, prompted patients to numerically indicate pain intensity, if present, after the anesthesia wore off and after 24 hours, and if there was a need to use systemic medication for both sessions. The pain level from 0 to 10 was divided into the following intervals: 0–2 (mild), 3–7 (moderate), and 8–10 (severe).

Statistical analysis

The data collected were entered into Excel spreadsheets (Microsoft in Redmond, WA) and analysed statistically using the software GraphPad Prism (GraphPad Software, LLC, CA, USA) at a significance level of 5%.

For the analysis of the number of bacterial species between MIC groups, Dunn's multiple comparisons test was used. To compare LPS levels between MIC groups, the Kruskal-Wallis test was employed, and for a comparison within the same group, Dunn's multiple comparisons test was used.


Enrollment
Assessed for eligibility (n = 88)
Excluded (n = 2)
Not meeting inclusion criteria (n = 45)
In the evaluation phase (n = 3)
Other reasons (n = 9)
Randomized (n = 29)
Allocation
Group 1:
Ca(OH)2 + saline solution
Group 2:
Ca(OH)2 + NAC
Group 3:
NAC + CHX
Allocated to intervation (n = 11)
Received allocated intervention (n = 11)
Did not received allocated intervention (n = 0)
Allocated to intervation (n = 9)
Received allocated intervention (n = 8)
Did not received allocated intervention (n = 1)
Allocated to intervation (n = 12)
Received allocated intervention (n = 11)
Did not received allocated intervention (n = 1)
Checkerboard analysis
Analysed (n = 8)
Not yet analyzed (n = 3)
Excluded from analysis (n = 0)
Analysed (n = 7)
Not yet analyzed (n = 1)
Excluded from analysis (n = 0)
Analysed (n = 6)
Not yet analyzed (n = 5)
Excluded from analysis (n = 0)
Quantification of Endotoxins
Analysed (n = 6)
Not yet analyzed (n = 5)
Excluded from analysis (n = 0)
Analysed (n = 8)
Not yet analyzed (n = 0)
Excluded from analysis (n = 0)
Analysed (n = 11)
Not yet analyzed (n = 0)
Excluded from analysis (n = 0)
Signs and symptoms
Analysed (n = 11)
Not yet analyzed (n = 0)
Excluded from analysis (n = 0)
Analysed (n = 8)
Not yet analyzed (n = 0)
Excluded from analysis (n = 0)
Analysed (n = 10)
Not yet analyzed (n = 0)
Excluded from analysis (n = 1)

Results

The resulting data of the checkerboard DNA-DNA analysis revealed that the presence of bacterial DNA was detected in 100% (21/21) of the samples collected from the root canals investigated. After instrumentation, the presence of bacterial DNA was detected in 95,23% (20/21) of the samples collected from the root canals investigated. After medication, the presence of bacterial DNA was detected in 100% (21/21) of the samples collected from the root canals investigated.

The median number of bacterial species per root canal found in S1 was 35 (range, 4–39), in S2 was 33 (range, 1–34), and in S3 was 39 (range, 0–39) (Table 1).

A total of 511 DNA strains belonging to 40 microbial species were recovered from 21 patients.

The most prevalent Gram-negative bacterial species detected in S1 were Prevotella melahinogenica (21/21 - 100%), Neisseria mucosa (18/21 - 85,71%) and Treponema denticola (18/21 - 85,71%), while the most prevalent Gram-positive bacterial species in S1 were Streptococcus sanguinis (19/21 - 90,47%), Streptococcus mitis (16/21 - 76,19%) and Propionybacterium acnes (16/21 - 76,19%) (Figure 1A).

In S2, the most prevalent Gram-negative bacterial species detected were Prevotella melahinogenica (18/21 - 85,71%), Neisseria mucosa (16/21 - 76,19%) and Treponema denticola (15/21 - 71,42%), whereas the most prevalent Gram-positive bacterial species were Streptococcus sanguinis (18/21 - 85,71%), Propionybacterium acnes (15/21 - 71,42%) and Streptococcus mitis (14/21 - 66,66%) (Figure 1B).

And, in S3, the most prevalent gram-negative bacterial species detected were Treponema denticola (16/21 - 76,19%), Prevotella melahinogenica (15/21 - 71,42%), and Fusobacterium nucleatum (14/21 - 66,66%), while the most prevalent gram-positive bacterial species were Propionybacterium acnes (14/21 - 66,66%), Streptococcus sanguinis (14/21 - 66,66%) and Actinomyces israelli (13/21 - 61,90%) (Figura 1C).

A statistical difference was observed between S1 and S2 regarding the median number of microbial species, a statistically significant difference was also observed from S1 to S3, however, no statistically significant difference was found from S2 to S3.

In an analysis stratified by MIC groups and different sampling times of the root canal, in S1, for the $Ca(OH)_2$ + saline solution group, the most commonly found Gram-negative bacterial species were Prevotella melahinogenica (8/8 - 100%), Prevotella intermedia (6/8 - 75%), Fusobacterium nucleatum (6/8 - 75%), while the most prevalent Gram-positive species were Streptococcus Constellatus (8/8 - 100%), Streptococcus sanguinis (7/8 - 87,5%) (Figure 2A). For the group $Ca(OH)_2$ + NAC, the most commonly found Gram-negative bacterial species in S1 were Prevotella melahinogenica (7/7 - 100%), Neisseria mucosa (7/7 - 100%) and Prevotella nigrescens (7/7 - 100%), whereas the most prevalent Gram-positive bacterial species were Streptococcus intermedius (7/7 - 100%), Actinomyces israelli (7/7 - 100%), and Streptococcus mitis (6/7 - 85,71%). And for the group (NAC + CHX), the most prevalent Gram-negative bacterial species in S1 were Capnocytophaga sputigena (6/7 - 85,71%), Prevotella melahinogenica (6/7 - 85,71%) and Treponema denticola (6/7 - 85,71%), and the most prevalent Gram-positive bacterial were Streptococcus sanguinis (6/7 - 85,71%), Propionybacterium acnes (5/7 - 71,42%) and Actinomyces israelli (5/7 - 71,42%) (Figure 3A). For the group NAC + CHX, the most commoly found Gram-negative bacterial species in S1 were Capnocytophaga sputigena (6/6 - 100%), Prevotella melahinogenica (6/6 - 100%), and Treponema denticola (6/6 - 100%), while the most prevalent Gram-positive bacterial species were Streptococcus sanguinis (6/6 - 100%), Propionybacterium acnes (5/6 - 83,33%), and Actinomyces israelli (5/6 - 83,33%) (Figure 4A).

For S2, for the $Ca(OH)_2$ + saline solution group, the most commonly found Gram-negative bacterial species were Prevotella melahinogenica (7/8 - 87,5%), Neisseria mucosa (6/8 - 85,71%) and Treponema denticola (5/8 - 62,5%), whereas the most commonly found Gram-positive bacterial species were Streptococcus sanguinis (8/8 - 100%), Propionybacterium acnes (6/8 - 85,71%), and Streptococcus mitis (5/8 - 62,5%) (Figure 2B). For the group $Ca(OH)_2$ + NAC, the most commoly found Gram-negative bacterial species in S2 were Prevotella melahinogenica (6/7 - 85,71%), Neisseria mucosa (6/7 - 85,71%), and Treponema denticola (6/7 - 85,71%), while the most commoly Gram-positive found bacterial species found were Streptococcus mitis (6/7 - 85,71%), Actinomyces israelli (6/7 - 85,71%), and Streptococcus sanguinis (6/7 - 85,71%) (Figure 3B). For the group NAC + CHX, the most commoly found Gram-negative bacterial species in S2 were Capnocytophaga

sputigena (5/6 - 83,33%), Prevotella melahinogenica (5/6 - 83,33%), and Fusobacterium nucleatum (4/6 - 66,66%), while the most commoly Gram-positive found bacterial species found were Streptococcus mitis (4/6 - 66,66%), Propionybacterium acnes (4/6 - 66,66%), and Actinomyces israelli (4/6 - 66,66%) (Figure 4B).

And, for S3, for the $Ca(OH)_2$ + saline solution group, the most commonly found Gram-negative bacterial species were Prevotella melahinogenica (4/8 - 50%), Fusobacterium nucleatum (4/8 - 50%), and Neisseria mucosa (4/8 - 50%), whereas the most prevalent Gram-positive bacterial species found were Streptococcus sanguinis (6/8 - 75%), Streptococcus Constellatus (6/8 - 75%), and Propionybacterium acnes (4/8 - 50%) (Figure 2C). For the group $Ca(OH)_2$ + NAC, the most commoly found Gram-negative bacterial species in S3 were, Prevotella nigrescens (7/7 - 100%), Prevotella melahinogenica (6/7 - 85,71%), and Fusobacterium nucleatum (6/7 - 85,71%), whereas the most prevalent Gram-positive bacterial species found were Propionybacterium acnes (7/7 - 100%), Actinomyces israelli (6/7 - 85,71%), and Streptococcus intermedius (5/7 - 71,42%) (Figure 3C). For the group NAC + CHX, the most commoly found Gram-negative bacterial species in S3 were Treponema denticola (6/6 - 100%), Prevotella intermedia (5/6 - 83,33%), and Capnocytophaga sputigena (5/6 - 83,33%), whereas the most prevalent Gram-positive bacterial species found were Actinomyces israelli (6/6 - 100%), Streptococcus sanguinis (6/6 - 100%), and Streptococcus mitis (5/6 - 83,33%) (Figure 4C).

Table 1 - *Descriptive statistics of the different root canal samples and MIC groups.*

	n	***Root canal samples***	***Median (minimum-maximum)***
Total samples	*21*	*S1*	*35,00 (4,0 – 39,00)*
		S2	*33,00 (1,00 – 34,00)*
		S3	*39,00 (0 – 39,00)*
$Ca(OH)_2$ + saline solution	*8*	*S1*	*35,00 (4,00 – 39,00)*
		S2	*24,00 (1,00 – 25,00)*
		S3	*30,00 (0 – 30,00)*
$Ca(OH)_2$ + NAC	*7*	*S1*	*28,00 (11,00 – 39,00)*
		S2	*21,00 (3,00 – 24,00)*
		S3	*18,00 (6,00 – 24,00)*
NAC + CHX	*6*	*S1*	*26,00 (12,00 – 38,00)*
		S2	*31,00 (3,00 – 34,00)*
		S3	*34,00 (5,00 – 39,00)*

Figure 1 - *The frequency and DNA concentration of individual bacterial species investigated in A) S1; B) S2; and C) S3.*

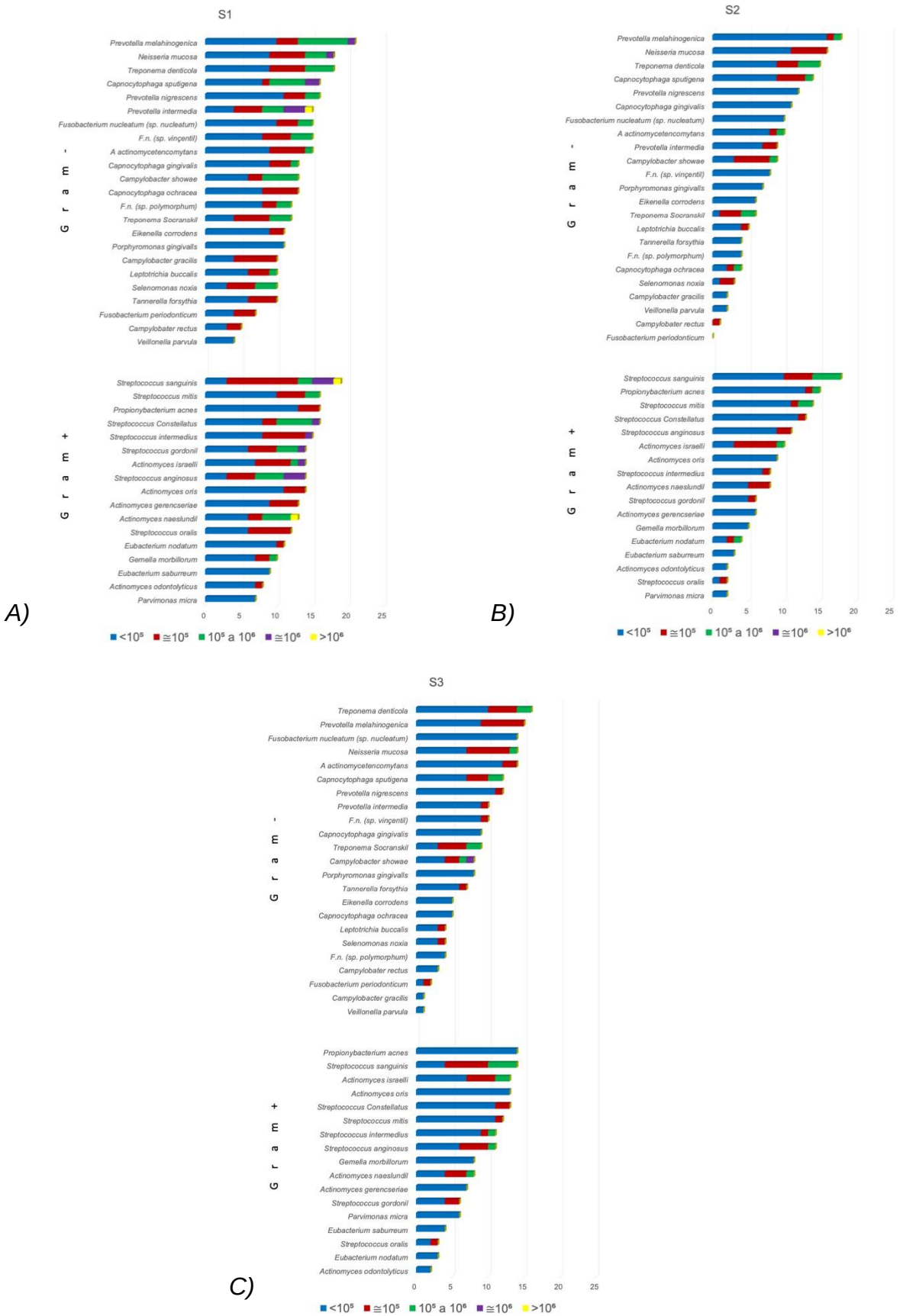


Figure 2 - *The frequency and DNA concentration of individual bacterial species investigated for the $Ca(OH)_2$ + saline solution group in A) S1; B) S2; and C) S3.*

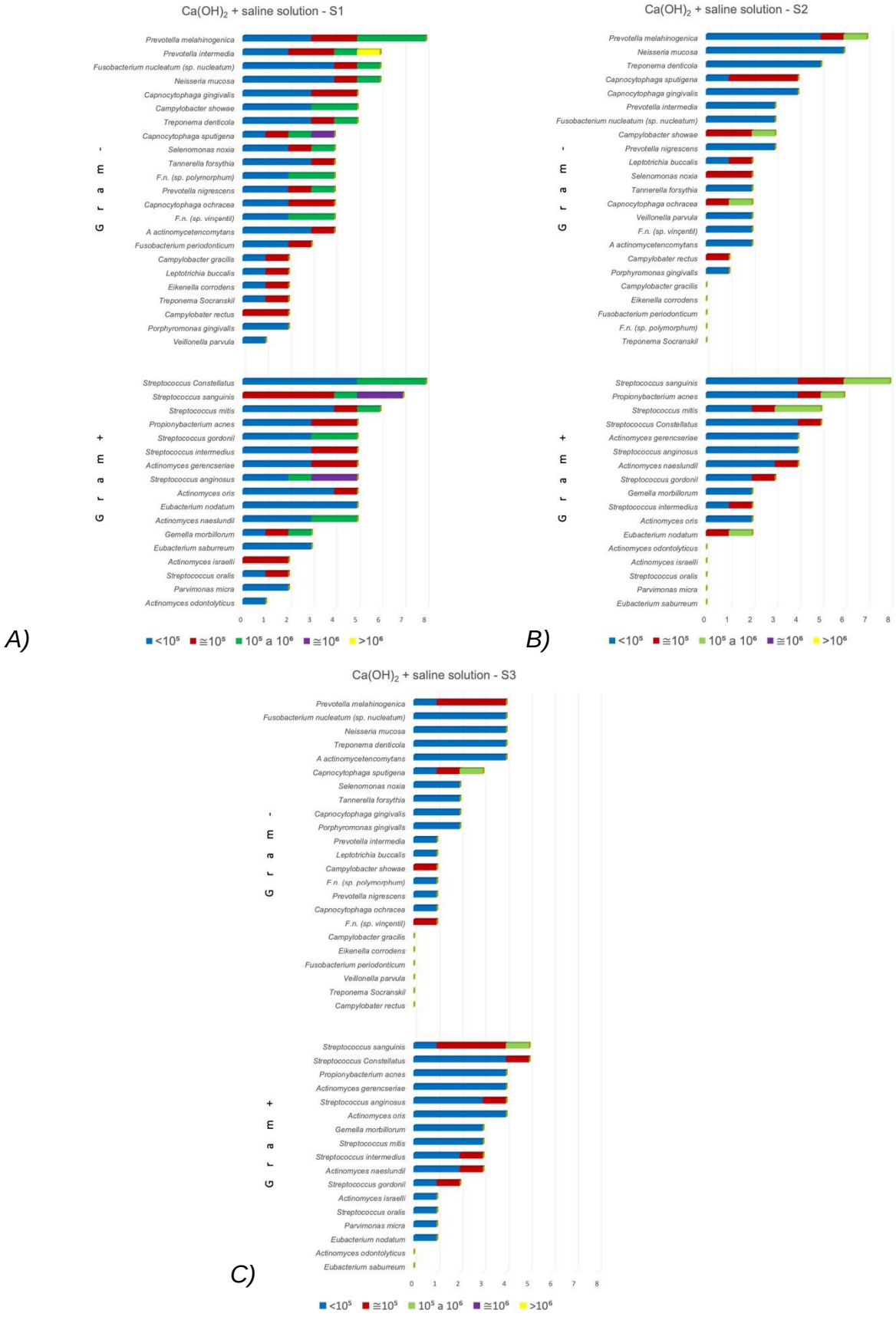


Figure 3 - *The frequency and DNA concentration of individual bacterial species investigated for the $Ca(OH)_2$ + NAC group in A) S1; B) S2; and C) S3.*

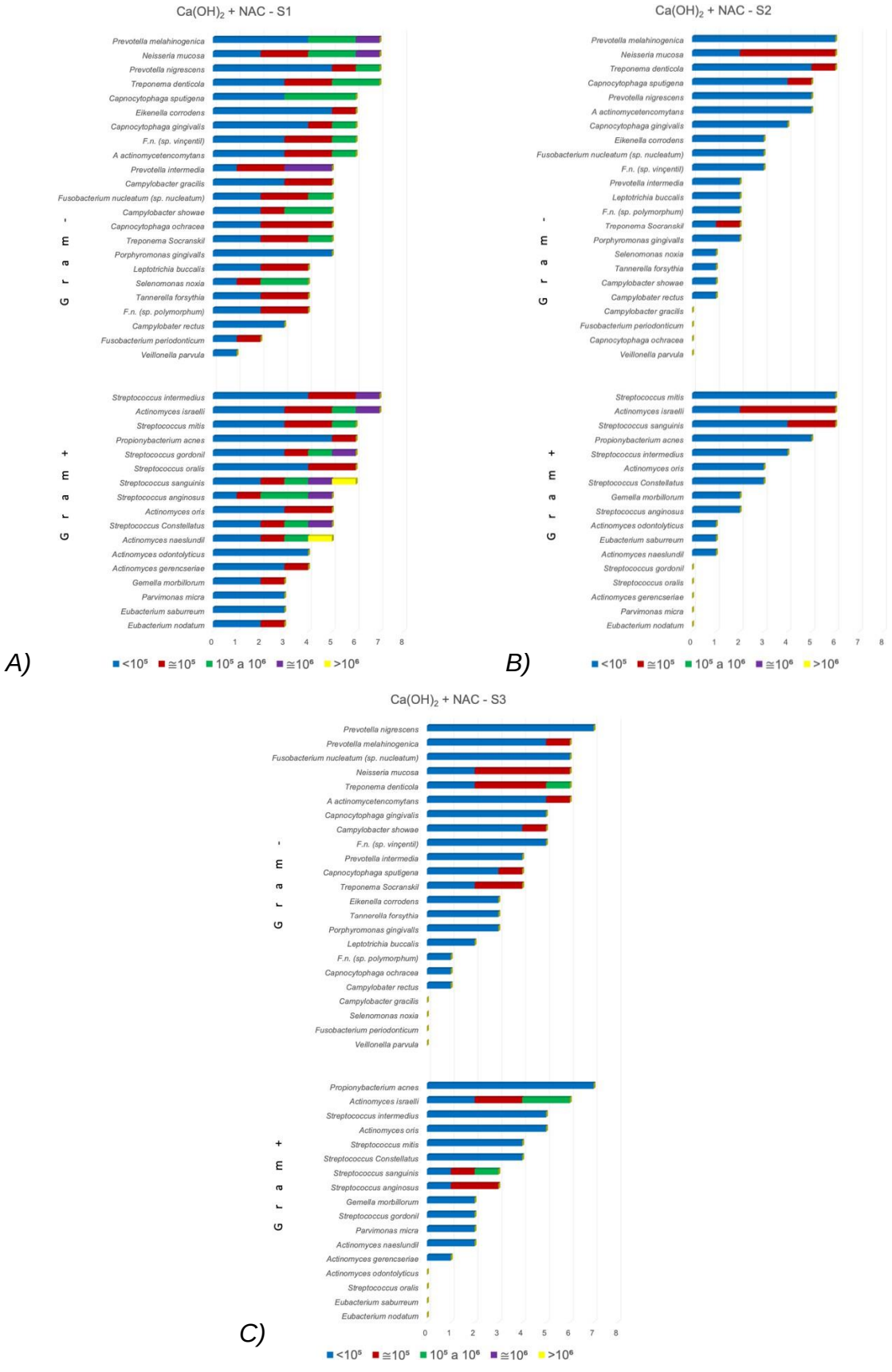
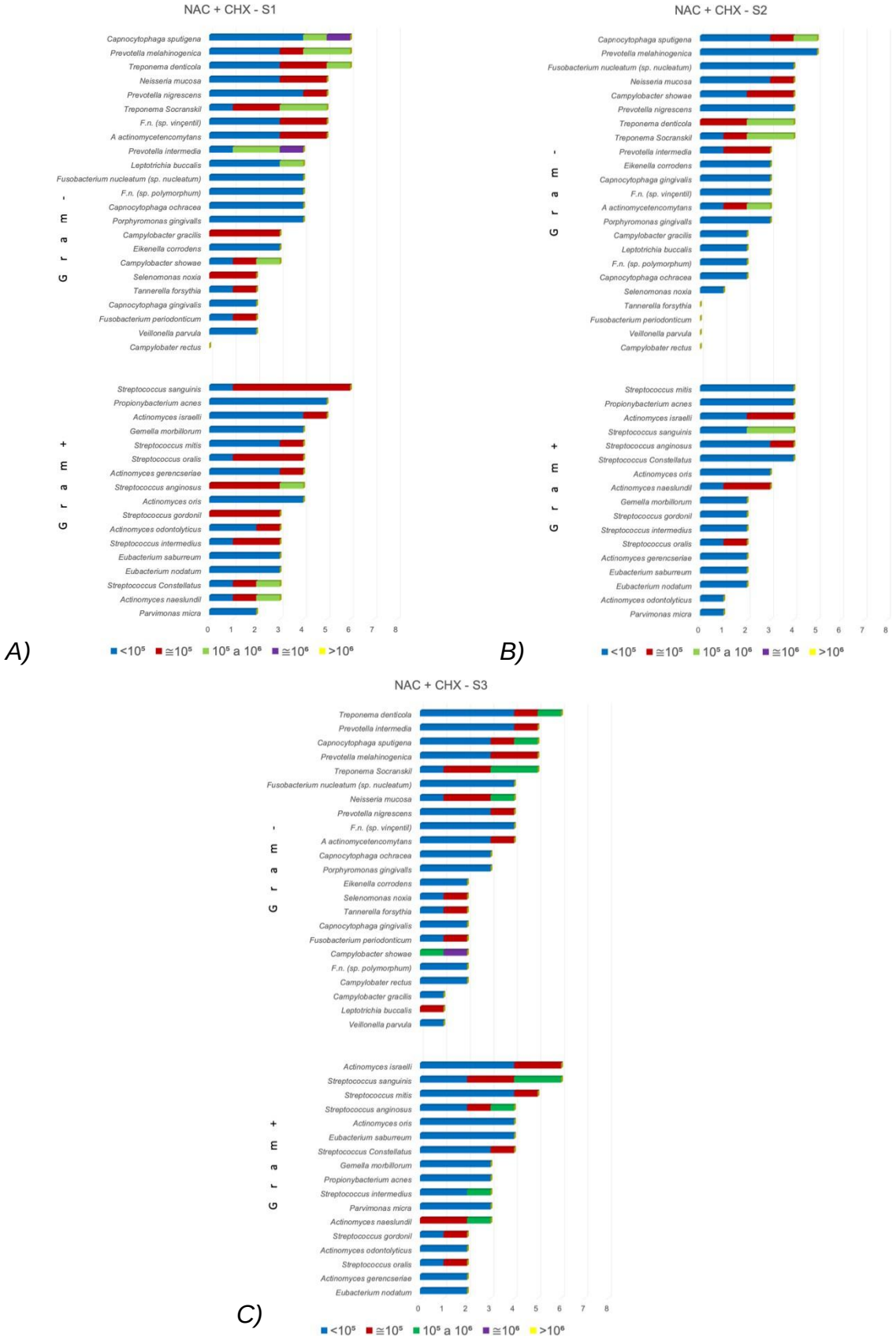


Figure 4 - *The frequency and DNA concentration of individual bacterial species investigated for the NAC + CHX group in A) S1; B) S2; and C) S3.*



Limulus Amebocyte Lysate (QCL-LAL) assay

No statistically significant difference was found between the medication groups (Table 2).

For the $Ca(OH)_2$ + saline solution group, no statistical difference was observed between S1 and S2 regarding the median number of LPS, however a statistically significant difference was observed from S1 to S3 nevertheless, and no statistically significant difference was found from S2 to S3 (Table 2). There was a 79.26% reduction from S1 to S2, a 94.31% reduction from S1 to S3, and a 72.57% reduction from S2 to S3.

For the $Ca(OH)_2$ + NAC group, a statistical difference was observed between S1 and S2 regarding the median number of LPS, however, no statistically significant difference was also observed from S1 to S3, and also, no statistically significant difference was found from S2 to S3 (Table 2). The data analysis revealed a 95.08% reduction from S1 to S2 and a 94.97% reduction from S1 to S3, whereas there was an increase of 2.27% from S2 to S3.

And, for the group NAC + CHX, a statistical difference was observed between S1 and S2 regarding the median number of LPS, a statistically significant difference was also observed from S1 to S3, nevertheless, no statistically significant difference was found from S2 to S3 (Table 2). The examination of the data indicated a decrease of 95.11% from S1 to S2 and a decrease of 97.96% from S1 to S3. In contrast, there was an increase of 58.41% from S2 to S3. The examination of the data indicated a decrease of 95.11% from S1 to S2 and a decrease of 97.96% from S1 to S3. In contrast, there was an increase of 58.41% from S2 to S3.

Table 2 - *Median (minimum and maximum value) of LPS (EU/mL) according to the group and phase of the endodontic treatment.*

	S1	***S2***	***S3***
$Ca(OH)_2$ + saline solution	*53,95 (6,70 – 155,00) Aa*	*11,19 (1,66 – 26,80) Aab*	*3,07 (0,38 – 8,58) Ab*
$Ca(OH)_2$ + NAC	*142,9 (5,73 – 443,30) Aa*	*7,04 (1,56 – 16,94) Ab*	*7,20 (0,33 – 27,27) Aab*
NAC + CHX	*198,60 (5,84 – 649,20) Aa*	*9,73 (1,05 – 39,25) Ab*	*4,06 (0,54 – 16,65) Ab*

Different letters indicate significant differences ($P < 0.05$). Different uppercase letters indicate a significant difference between the groups. Different lowercase letters indicate a significant difference within the same group.

Signs and symptoms

The following clinical features were found before the treatment: spontaneous pain (9/29), swelling (5/29), fistula (8/29), sensitivity to percussion (13/29) and sensitivity to palpation (13/29). After 14 days of the first session and MIC the following clinical features were found before the treatment: spontaneous pain (0/29), swelling (0/29), fistula (1/29), sensitivity to percussion (1/29) and sensitivity to palpation (2/29).

In a stratified analysis by groups, for the $Ca(OH)_2$ + saline solution group, the following clinical features were found before the treatment: spontaneous pain (4/11), swelling (2/11), fistula (3/11), sensitivity to percussion (6/11), and sensitivity to palpation (6/11). After 14 days of the first session and MIC, the following clinical features were observed: spontaneous pain (0/11), swelling (0/11), fistula (1/11), sensitivity to percussion (0/11), and sensitivity to palpation (0/11). After the first session, the following pain symptoms were reported: after the anesthesia wore off, (1/11) experienced severe pain, (1/11) experienced moderate pain, and after 24 hours, (1/11) had mild pain and (2/11) needed medication. After the second session, the following pain symptoms were reported: after the anesthesia wore off, (1/11) experienced moderate pain, and after 24 hours, (3/11) experienced moderate pain, and 1 patient needed systemic medication.

For the $Ca(OH)_2$ + NAC group, the following clinical features were found before the treatment: spontaneous pain (3/8), swelling (1/8), fistula (1/8), sensitivity to percussion (3/8), and sensitivity to palpation (3/8). After 14 days of the first session and MIC, the following clinical features were observed: spontaneous pain (0/8), swelling (0/8), fistula (0/8), sensitivity to percussion (1/8), and sensitivity to palpation (1/8). After the first session, the following pain symptoms were reported: after the anesthesia wore off, (3/8) experienced moderate pain, and after 24 hours, (1/8) experienced severe pain, (2/8) experienced mild pain, and (3/8) needed medication. After the second session, the following pain symptoms were reported: after the anesthesia wore off, (1/8) experienced moderate pain, and after 24 hours, (1/8) experienced moderate pain, and 1 patient needed systemic medication.

For the NAC + CHX group, the following clinical features were found before the treatment: spontaneous pain (2/10), swelling (2/10), fistula (4/10), sensitivity to percussion (4/10), and sensitivity to palpation (4/10). After 14 days of the first session and MIC, the following clinical features were observed: spontaneous pain (0/10), swelling (0/10), fistula (0/10), sensitivity to percussion (0/10), and sensitivity to palpation (1/10). After the first session, the following pain symptoms were reported: after the anesthesia wore off, (1/10) experienced mild pain, (2/10) experienced moderate pain, and (1/10) experienced severe pain, and after 24 hours, (1/10) experienced mild pain, (3/10) experienced moderate pain, and (3/10) needed medication. After the second session, the following pain symptoms were reported: after the anesthesia wore off, (0/10) experienced pain, and after 24 hours, (0/10) experienced pain, and 0 patients needed systemic medication.

There was no correlation found between bacterial species and the manifestation of signs and symptoms.

Discussion

The data obtained from checkerboard DNA-DNA hybridization indicated that all initial samples (S1) exhibited an average of 35 species per root canal, and the most prevalent Gram-negative bacterial species were Prevotella melahinogenica (21/21 - 100%), Neisseria mucosa (18/21 – 85,71%) and Treponema denticola (18/21 – 85,71%), while the most prevalent Gram-positive bacterial species in S1 were

Streptococcus sanguinis (19/21 – 90,47%), Streptococcus mitis (16/21 – 76,19%) and Propionybacterium acnes (16/21 – 76,19%), confirming the polymicrobial nature of primary endodontic infections, which comprise a mixed community of both Gram-positive and Gram-negative bacteria (1–4).

Other authors identified the bacterial composition using the checkerboard DNA-DNA hybridization method (1, 40), Cavalli et al. (2017) (1) reported an average of 11,3 initial bacterial species per root canal (S1), while Machado et al. (2020) (40) found an average of 9 species per canal. Both studies (Cavalli et al. (2017) (1) and Machado et al. (2020) (40)) employed VMGA III as the transport medium for samples collected from inside the root canals, which may have an impact on DNA viability for checkerboard DNA-DNA hybridization analysis.

Additionally, different studies regarding the composition of endodontic microbiota vary among authors (1, 40). According to Siqueira (2002) (41), any bacteria colonizing the necrotic pulp may contribute to the pathogenesis of periapical disease. However, not all are capable of being disease-causing agents, and only a limited number of species are more prevalent in different forms of the disease.

Regarding collection after biomechanical preparation (S2), unlike findings from other authors (1, 40), the mean number of bacterial species per canal remained the same after biomechanical preparation. However, the reduction from S1 to S2 was statistically significant, although the most prevalent bacterial species after biomechanical preparation (S2) did not coincide (1, 40).

A significant statistical difference was noted between S1 and S2 regarding the median count of microbial species. Furthermore, a statistically significant difference was observed from S1 to S3; however, no significant difference was found from S2 to S3. Following MIC with $Ca(OH)_2$ + saline solution, there was an increase in the median count from S2 to S3. Ferreira et al. (2015) (42) also observed an increase in bacterial count after intracanal medication with $Ca(OH)_2$ + saline solution.

In the present study, the most prevalent bacterium during treatment was Fusobacterium nucleatum (sp. nucleatum), which was also noted by other authors (42).

Regarding the bacterial species found after MIC for a period of 14 days (S3), Fusobacterium nucleatum (sp. nucleatum) was the most prevalent bacterium found in the $Ca(OH)_2$ + saline solution group in Ferreira et al.'s (2015) (41) study, and in the

present study, this bacterium was also among the most prevalent after S3 for the $Ca(OH)_2$ + saline solution and $Ca(OH)_2$ + NAC groups, suggesting that the association with chlorhexidine is capable of eliminating Fusobacterium nucleatum (sp. nucleatum).

Certain bacterial species (1), as well as higher levels of endotoxins (43), are directly related to the manifestation of clinical signs and symptoms. However, due to the low sample size in the present study, it was not possible to establish this relationship statistically. However, post-treatment, patients in the NAC + CHX group reported the least pain and need for systemic medication, suggesting better clinical efficacy of this intracanal medication.

Endotoxins serve as virulence factors that play a crucial role in perpetuating endodontic disease (5,7,10,43). Clinically, endotoxins derived from bacteria involved in endodontic infections are closely linked to the process of periapical bone resorption, demonstrating a direct relationship between the volume of periapical lesions and the quantity of endotoxins present in the root canals (44). Endodontic treatment generally aims to maximize the reduction of microorganisms and endotoxins in the root canals to levels compatible with healing. Therefore, the stage of biomechanical preparation, involving the mechanical action of instruments combined with irrigating solutions, allows for a significant decrease of endotoxins (44). Furthermore, the agitation of the irrigating solution enables an even greater reduction of endotoxins in the root canal system (46), and the use of intracanal medication (MIC) between appointments allows for the neutralization of the cytotoxic effects of endotoxins (5,10).

It was found that in the $Ca(OH)_2$ + saline solution group, there was no significant reduction of LPS after biomechanical preparation. However, the intracanal medication further reduced LPS levels, leading to a difference between S3 and S1. This suggests that the MIC was crucial for endotoxin neutralization in S3. As for the $Ca(OH)_2$ + NAC group, there were no significant reductions in LPS levels, whereas the NAC + CHX group significantly reduced bacterial LPS.

Conclusion

Thus, considering the limitations, especially regarding the number of samples

of this study, it was observed that concerning bacterial species, LPS, and clinical signs and symptoms, NAC combined with $Ca(OH)_2$ or CHX showed similar results to the control group ($Ca(OH)_2$ + saline solution), which is already a well-established MIC in Endodontics. Therefore, it is essential to increase the sample size in this study to obtain more significant results.

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2.2 Artigo – Fiamini, BK; Santos, AC; Guerrero, GG, Gagliardi, CF; Barbosa, CGC; Valera, MC. Correlação entre Perfil Inflamatório e Volume de Lesões Periapicais em Infecções Endodônticas Primárias / *Correlation between Inflammatory Profile and Volume of Periapical Lesions in Primary Endodontic Infections*.

RESUMO

Citocinas são fundamentais na regulação da resposta inflamatória, essas moléculas facilitam a ativação e diferenciação de osteoclastos, células responsáveis pela reabsorção óssea na área apical. Este estudo investigou a correlação entre citocinas inflamatórias (IL-1β, IL-6, IL-17a e TNF-α) e o volume de destruição periapical em dentes unirradiculares com infecções endodônticas primárias. Foram selecionados vinte e nove dentes unirradiculares com infecção endodôntica primária e lesão periapical. Amostras de fluido periapical foram coletadas e analisadas por meio de multiplex, para quantificação de citocinas. O volume das lesões periapicais foi medido utilizando tomografia computadorizada de feixe cônico (CBCT) e analisado com o software ITK-Snap. Os dados foram tabulados e analisados estatisticamente. O teste de correlação de Spearman demonstrou uma correlação positiva entre o volume de destruição periapical e IL-1β (correlação moderada) e TNF-α (correlação fraca), no entanto, não mostrou uma correlação entre IL-6 e IL-17. Esses achados sugerem que IL-1β e TNF-α influenciam diretamente no processo de reabsorção óssea periapical.

Palavras-chave: Citocinas; Endodontia; Periodontite Periapical; Tomografia Computadorizada de Feixe Cônico.

ABSTRACT

Cytokines are fundamental in regulating the inflammatory response, as these molecules facilitate the activation and differentiation of osteoclasts, the cells responsible for bone resorption in the apical area. This study investigated the correlation between inflammatory cytokines (IL-1β, IL-6, IL-17a, and TNF-α) and the volume of periapical destruction in single-rooted teeth with primary endodontic infections. Twenty-nine single-rooted teeth with primary endodontic infection and periapical lesions were selected. Periapical fluid samples were collected and analyzed using multiplex for cytokine quantification. The volume of the periapical lesions was measured using cone-beam computed tomography (CBCT) and analyzed with ITK-Snap software. The data were tabulated and statistically analyzed. Spearman's correlation test demonstrated a positive correlation between the volume of periapical destruction and IL-1β (moderate correlation) and TNF-α (weak correlation); however, it did not show a correlation between IL-6 and IL-17. These findings suggest that IL-1β and TNF-α directly influence the process of periapical bone resorption.

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Keywords: Cone-Beam Computed Tomography; Cytokines; Endodontics; Periapical Periodontitis.

Introduction

Cytokines and chemokines are key proteins involved in cellular signaling and are vital for regulating immune responses in primary endodontic infections, these proteins influence processes such as lymphocyte activation, proliferation, differentiation, survival, and apoptosis (1,2). They are secreted by different cell types within damaged tissues and play a pivotal role in regulating cellular activity during inflammation, this modulation occurs through either altering cellular functions or recruiting defense cells to the site of injury (3). Moreover, cytokines are categorized into pro-inflammatory types that amplify cellular immune responses and anti-inflammatory types that promote antibody responses (1).

In conjunction with prostaglandins, cytokines such as interleukins and tumor necrosis factor alpha (TNF-α) are pivotal in regulating the inflammatory response. These molecules facilitate the activation and differentiation of osteoclasts, the cells responsible for bone resorption in the apical area (4).

Interleukin 1β (IL-1β) plays a significant role in the resorption of periradicular lesions (5–7). Its presence is correlated with the extent of periapical bone destruction (8), and the manifestation of clinical signs and symptoms (6). Similarly, interleukin 6 (IL-6), a pro-inflammatory cytokine, plays a crucial role in bone remodeling and in the activation and differentiation of immune system cells and osteoclasts. IL-6 is found in more advanced stages of periapical lesions (9). However, in the absence of IL-6, other cytokines such as IL-1β and TNF-α can induce bone resorption (10,11).

TNF-α is a well-established cytokine known as a key mediator of the acute inflammatory response (12). Additionally, TNF-α influences stromal cells by elevating the expression of factors linked to bone resorption and facilitating osteoclast differentiation, this occurs even when levels of proteins involved in the regulation and differentiation of osteoclasts, such as receptor activator of nuclear factor kappa-B ligand (RANKL), are not elevated (12). Both TNF-α and RANKL, either alone or in conjunction, significantly influence the differentiation of osteoclasts (13,14).

Interleukin-17 (IL-17) is a member of a newly recognized family of pro-

inflammatory cytokines predominantly produced by a recently acknowledged subset of activated T cells known as "Th17" cells (15,16). IL-17 was first identified as a pro-inflammatory cytokine because of its significant role in defending the host against extracellular bacterial and fungal infections (17,18). However, excessive production of IL-17 can lead to chronic inflammation linked to various inflammatory and autoimmune conditions (19). In combination with collagenases, IL-17 also stimulates the release of antimicrobial peptides and chemokines that attract neutrophils, thereby sustaining persistent infection (17,20). Therefore, the effects of IL-17 signaling can be either beneficial or harmful depending on both the level of IL-17 produced and the manner in which IL-17 signals are received and processed within the responding cell (19).

In Endodontics, cone-beam computed tomography (CBCT) is frequently used because it provides three-dimensional (3D) images with exceptional detail and high resolution (21–23). Hypodense images surrounding the root apices indicate a potential endodontic origin of disease (24), in such instances, differentiating apical periodontitis is crucial for establishing an effective treatment plan to manage endodontic infections (24–26). CBCT is the imaging modality that offers the greatest accuracy in diagnosing apical periodontitis (24–27), allowing the detection of the disease in its early stages (24).

Therefore, the aim of this study was to examine the relationship between the inflammatory profile of primary endodontic infections and the volume of periapical lesions.

Materials and methods

The research underwent evaluation by the Ethics Research Committee at the Institute of Science and Technology of São José dos Campos (ICT-SJC) of UNESP and received approval under review number 5.828.002. Following this, the study will be registered with the Brazilian Registry of Clinical Trials (REBEC).

Patient selection and eligibility criteria

Twenty-nine teeth were selected for the study based on the following criteria:

(1) single-rooted teeth; (2) the presence of periapical lesions; and (3) primary endodontic infections. These criteria were verified using periapical radiographs and CBCTs.

The exclusion criteria were as follows: (1) teeth with prior endodontic treatment; (2) teeth exhibiting periodontal disease; (3) teeth with root fractures; (4) patients who had used antifungal or antibiotic medications within three months prior to initiating endodontic treatment; (5) cases where rubber dam isolation could not be achieved; and (6) cases where the insertion of the paper point to the working length (WL) of the root canal was not feasible. Patients were recruited from June 2023 to April 2024.

Clinical procedures and collection

Patients underwent anesthesia and the teeth was isolated with rubber dam. The crown of the tooth was cleaned with saline solution (Laboratório Sanobiol, Pouso Alegre, MG, Brazil) and the interface between the crown and the work area was sealed with Top Dam light-cured resin barrier (FGM Produtos Odontológicos, Joinville, SC, Brazil). Prophylaxis of the crown was performed with prophylactic paste (Vigodent S/A Indústria e Comércio, Rio de Janeiro, RJ, Brazil) and Robinson brush (Microdont – Micro Usinagem de Precisão Ltda, São Paulo, SP, Brazil), followed by disinfection of the surgical area with sterile swabs (Jiangsu Medical Materials Co. Ltd., Jiangsu, China) soaked in 30-volume hydrogen peroxide (Byofórmula, São José dos Campos, SP, Brazil) and 5.25% sodium hypochlorite (NaOCl) (Byofórmula, São José dos Campos, SP, Brazil). Subsequently, NaOCl was neutralized with 5% sodium thiosulfate (Byofórmula, São José dos Campos, SP, Brazil).

Access to the pulp chamber was performed under sterile saline irrigation. Once access was gained, biomechanical preparation was performed using the crown-down technique with the Reciproc® reciprocating file system (VDW, Munich, Germany). Files were selected based on the diameter of the K-file that best fit the root canal; if the K-file #20 fit, the R40 file was used, and if the K-file #30 was appropriate, the R50 file was used. WL was determined using the RomiApex locator (RomiBras LTDA, Rio de Janeiro, RJ, Brazil) and confirmed by radiography. WL was set to 1 mm short of the apical foramen.

Instrumentation was divided into thirds (cervical, middle, and apical) and was accompanied by irrigation with 2.5% NaOCl, using 8 mL for each third, totaling 24 mL overall. The canals were then irrigated with 17% EDTA for 3 minutes, agitating the solution with an ultrasonic tip Irrisonic 20/01 (Helse Dental Technology, Santa Rosa de Viterbo, SP, Brazil) 2 mm from the WL, repeating the process in three 20-second cycles. Each cycle was conducted with renewed EDTA solution, totaling 1 mL. Next, the canals were flooded with 2.5% NaOCl and subjected to ultrasonic agitation for three cycles of 20 seconds each. The solution was renewed between each cycle, totaling 5 mL of solution. To neutralize the NaOCl used in the final irrigation protocol, the canal was rinsed with 5 mL of sodium thiosulfate (Byofórmula, São José dos Campos, SP, Brazil) followed by an irrigation with 10 mL of sterile saline solution.

Collection of periapical fluid

For the collection of periapical fluid (SF), a manual K-file #30 was introduced into the canal, extending 2 mm beyond the WL of the tooth along with irrigation using 5 mL of sterile saline solution. The objective was to widen the apical foramen, allowing the insertion of paper points, which were inserted along the entire length of the root canal to collect the interstitial fluid from the periapical lesion. Three points were left in position for 60 seconds and then stored in a empty Eppendorf, and finally, they were stored at -80°C. After the SF, the tooth received intracanal medication.

Analysis of inflammatory cytokines by multiplex assay

Four inflammatory cytokines (IL-1β, IL-6, IL-17a, and TNF-α) were assessed via multiplex analysis using the MILLIPLEX® MAP Human Cytokine/Chemokine/Growth Factor Panel A kit (HCYTA-60K-04, Merck Life Science, LLC, Darmstadt, Germany), following the manufacturer's guidelines. The first step involved an 18-hour incubation of beads with the samples, with the assay plate kept at a constant temperature of 4°C, under agitation on the orbital shaker (600 rpm). After the samples were captured by the microspheres, biotinylated detection antibodies were added. Subsequently, this complex was conjugated with streptavidin-PE, resulting in complete reaction on the surface of each microsphere.

The plate containing the samples was analyzed using the Luminex MAGPIX System (Luminex Corporation, Austin, TX, USA), and the run was performed using xPONENT 4.3 software, with data processed using Belysa 1.2 analysis software (Merck Life Science). The MAGPIX System® equipment allowed for internal encoding of microspheres, which are stained with two fluorescent dyes, in addition to the identification process of each individual microsphere, and the bioassay results were quantified based on fluorescent signals. The final data were expressed as Mean Fluorescence Intensity (MFI).

Analysis of periapical bone destruction volume

CBCTs were conducted at the Department of Diagnosis and Surgery at the Institute of Science and Technology of São José dos Campos (ICT-SJC). The volume of periapical lesions was calculated according to the methodology described by Cardoso et al. (2015) (29). For the acquisition CBCT images, the patient's occlusal plane was positioned parallel to the horizontal plane (corresponding to the axial plane) in accordance with the manufacturer's recommended protocol. All scans were performed using the Classic I-Cat scanner (Imaging Science International, Hatchfield, USA) with a 6 cm field of view (FOV), an isotropic voxel size of 0.25 mm, 120 kVp, 36, 15 mAs, and a grayscale depth of 12 bits.

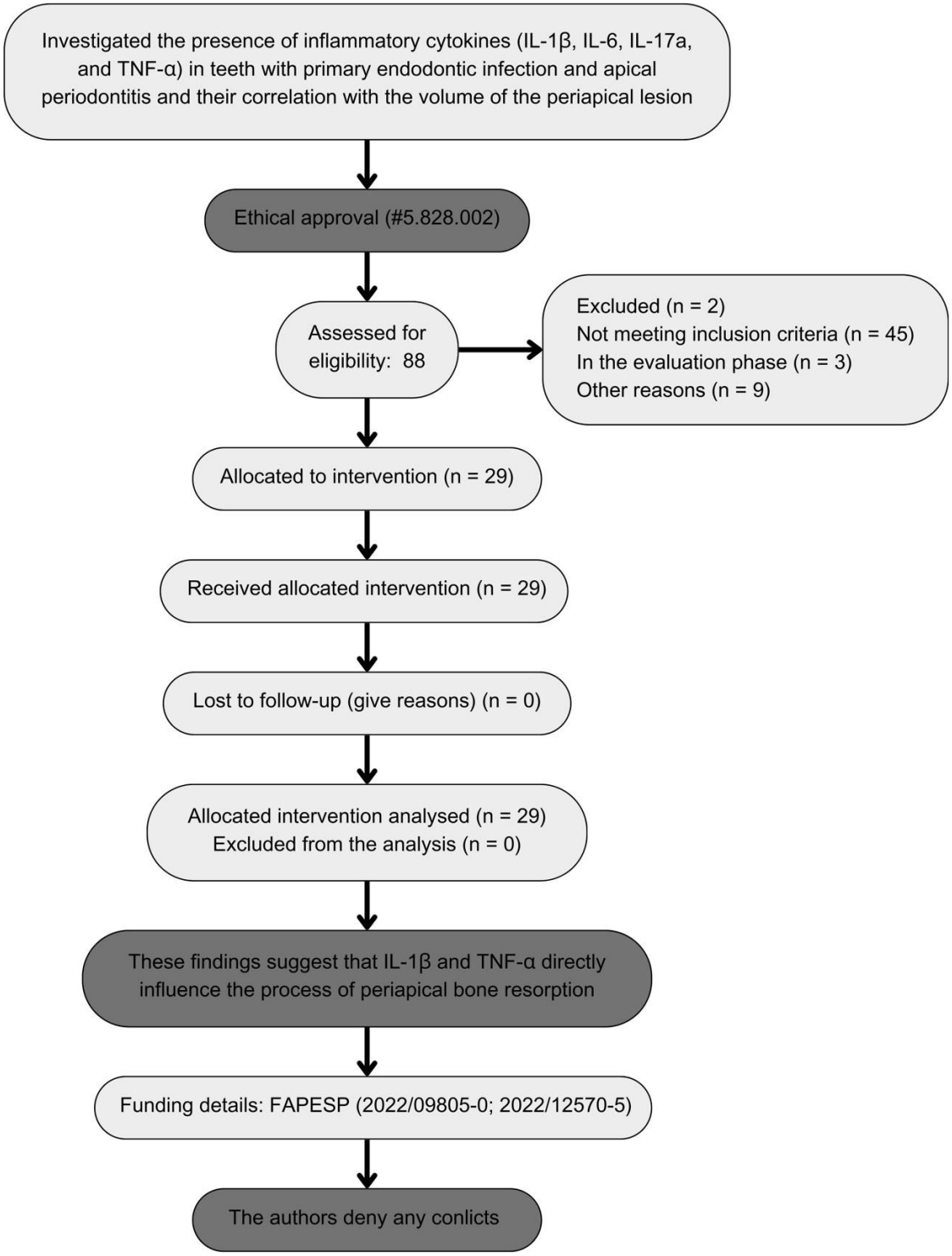
All exams were processed in DICOM (Digital Imaging and Communications in Medicine) format and interpreted using specialized software. The DICOM data from each scan were saved as separate files, then imported and analyzed using ITK-Snap software to calculate the volume of periapical radiolucency.

The segmentation and volumetric measurements were conducted by positioning the slice in each of the three anatomical planes (axial, sagittal, and coronal) to capture the full extent of periapical lesions. A polygon was then created to encompass the lesion boundary for 3D reconstruction using a threshold of 350 Hounsfield units, chosen based on consensus from a pilot study. Two-dimensional segmentation across all planes identified the radiolucent area, with careful validation of lesion boundaries. Subsequently, a 3D reconstruction was generated by volumetric expansion of selected areas across all slices. The software's "volume detect" feature automatically calculated volumes in cubic centimeters, later converted to cubic

millimiters.

Statistical analysis

The information gathered was inputted into Excel spreadsheets (Microsoft in Redmond, WA). Statitics were conducted by GraphPad Prism (GraphPad Software, LLC, CA, USA). The test of Spearman was used to analyse the correlation between the volume of periapical lesions and the quantity of inflammatory cytokines. A 90% confidence interval was adopted (p <0.1).


Investigated the presence of inflammatory cytokines (IL-1β, IL-6, IL-17a, and TNF-α) in teeth with primary endodontic infection and apical periodontitis and their correlation with the volume of the periapical lesion
Ethical approval (#5.828.002)
Assessed for eligibility: 88
Excluded (n = 2)
Not meeting inclusion criteria (n = 45)
In the evaluation phase (n = 3)
Other reasons (n = 9)
Allocated to intervention (n = 29)
Received allocated intervention (n = 29)
Lost to follow-up (give reasons) (n = 0)
Allocated intervention analysed (n = 29)
Excluded from the analysis (n = 0)
These findings suggest that IL-1β and TNF-α directly influence the process of periapical bone resorption
Funding details: FAPESP (2022/09805-0; 2022/12570-5)
The authors deny any conlicts

Results

A total of 29 teeth were included in the present study, in which the median volume of the samples was 36,5 mm^3 (Table 1). Inflammatory cytokines were detected in 100% of the periapical fluid samples, with median values of 15 MFI (IL-1β), 34 MFI (IL-6), 29 MFI (IL-17a), and 16 MFI (TNF-α) (Table 1). Spearman's

correlation test demonstrated a positive correlation between the volume of periapical destruction and IL-1β and TNF-α (Table 2 and Figure 1 and 4), however, it did not show a correlation between IL-6 and IL-17a (Table 2 and Figure 2 and 3).

Table 1 – *Median and range values of inflammatory cytokines and volume of periapical bone destruction.*

	Volume of bone destruction (mm^3)	***IL-1β (MFI)***	***IL-6 (MFI)***	***IL-17a (MFI)***	***TNF-α (MFI)***
N	*29*	*29*	*29*	*29*	*29*
Median	*36,50*	*15,00*	*34,00*	*29,00*	*16,00*
Minimum	*6,23*	*11,00*	*6,00*	*15,00*	*10,00*
Maximum	*340,00*	*157,00*	*2036,00*	*46,00*	*177,00*

Table 2 - *Correlation between periapical bone destruction volume and inflammatory cytokines.*

		IL-1β	***IL-6***	***IL-17a***	***TNF-α***
Volume of bone destruction	***r***	*0,4596*	*0,1265*	*0,2201*	*0,3359*
	p	*0,0121**	*0,5132*	*0,2512*	*0,0748**

(p<0.1)

Figure 1 – *XY correlation of IL-1β 90%*

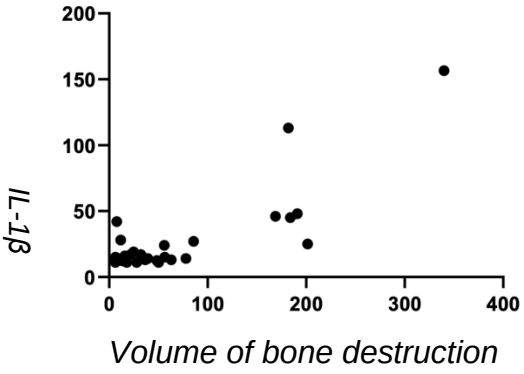


Figure 2 – *XY correlation of IL-6 90%*

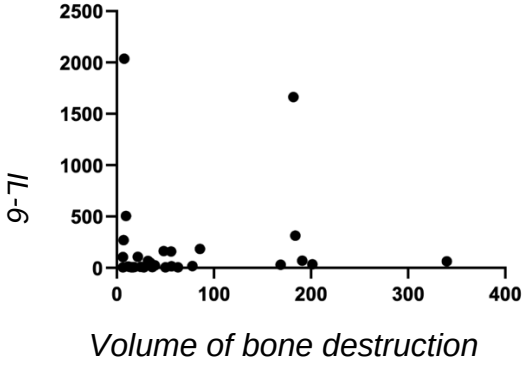


Figure 3 – *XY correlation of IL-17a 90%*

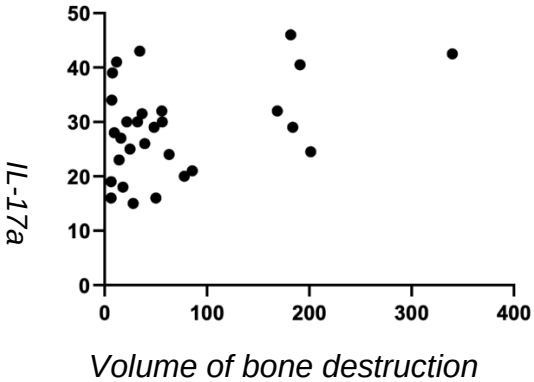
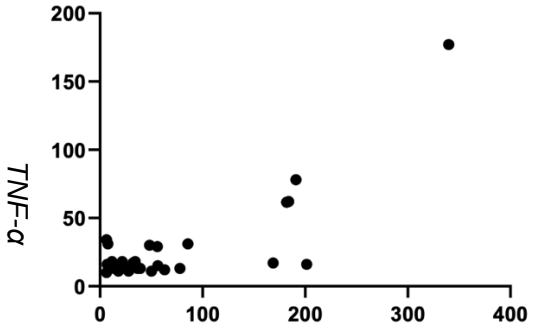


Figure 4 – *XY correlation of TNF-α a 90%*



Volume of bone destruction

Discussion

This study aimed to correlate the volume of periapical bone destruction with the levels of inflammatory cytokines in periapical fluid present in single-rooted teeth with primary endodontic infection. For the interstitial fluid collections in the present study, the methodology described by Corazza et al. (2021) and Martinho et al. (2016) (30) was employed, where a new instrumentation using a #30 file was performed, surpassing 1 mm of the working length (WL) of the tooth, followed by the insertion of an absorbent paper point. According to Virdee et al. (2019) (31), collection through absorbent paper points is the most effective and widely used in clinical research for periapical fluid collection; however, the quantity of periapical fluid collected in the present study was low, with many values below the standard 1 of the standard curve, therefore, the data were expressed in MFI. Given the complexity and importance of periapical fluid as an essential biomarker in endodontic conditions, there is a need to explore and establish new collection methods.

In this clinical research, a moderate correlation was found between the amount of IL-1β present in periapical fluid and the volume of periapical bone destruction. These findings align with the results obtained by Popovska et al. (2017) (32), who also reported a moderate correlation between the size of periapical lesions and the amount of IL-1β. Additionally, Popovska et al. (2017) (32) observed that IL-1β is present in chronic periapical lesions during acute exacerbation stages and in granulomas at early developmental stages. Bakhsh et al. (2022) (33) found higher levels of IL-1β and IL-6, which were also observed in larger periapical lesions. Compared to the present study, these findings are consistent with IL-1β but not with

IL-6, which did not show a correlation with the volume of periapical bone destruction.

Ataonlu et al. (2002) (34) evaluated the levels of IL-1β and TNF-α in periapical exudates collected using paper points and found higher levels of IL-1β compared to TNF-α, with greater amounts of IL-1β being associated with larger areas of periapical radiolucency. These findings corroborate the results of the present study, where IL-1β and TNF-α may play a pro-inflammatory role, leading to increased levels and bone resorption in the presence of infections.

In this study, we did not find a correlation between IL-6 and the volume of periapical bone destruction, Hofbauer et al. (1999) (35), found that TNF-α and IL-1β stimulate steady-state levels of osteoprotegerin ligand mRNA, a crucial factor for inducing osteoclastogenesis in various human osteoblastic lineage cells, while IL-6 did not demonstrate this effect. However, as suggested by Azuma et al. (2014) (10), during periapical lesion formation, there is a local increase in IL-6 in response to infection, which may induce the formation of new osteoclasts from hematopoietic progenitor cells, as well as activate pre-existing osteoclasts. This could theoretically establish a direct relationship between IL-6 and the volume of periapical lesions. However, these findings do not align with the results of this clinical study. Furthermore, in a clinical study conducted by Martinho et al. (2012) (36), the size of radiographically observed periapical lesions correlated with TNF-α and IL-6, but not with IL-1β. While the results for TNF-α corroborate with this study, the results for IL-6 and IL-1β did not find support.

According to the findings of the present study, IL-17a showed no correlation with the volume of periapical bone destruction. Additionally, among the cytokines studied in this research, IL-17a exhibited the lowest expression. Consistent with these findings, Ajuz et al. (2014) (37) reported no correlation between periapical lesion volume and IL-17a. Studies have reported (16,38) both protective and destructive roles of IL-17a in apical periodontitis, where it stimulates the production of inflammatory mediators promoting osteoclastogenesis and subsequent periapical bone destruction, moreover, IL-17a plays a crucial role in the mobilization and recruitment of neutrophils, thereby preventing infection-stimulated inflammatory bone resorption. However, no correlation was observed between IL-17a and the volume of periapical lesions. There is a lack of studies investigating the mechanism of action of IL-17a in apical periodontits, highlighting the necessity for further research.

In this study, it was observed that the amount of TNF-α present in periapical fluid correlates with the volume of periapical bone destruction; however, this correlation proved to be weak. TNF-α is a pro-inflammatory cytokine that acts on osteoclast differentiation, synergizing with RANKL (39), thus directly related to the process of periapical bone resorption.

It is noteworthy that IL-1β is 500 times more potent than TNF-α in mediating bone resorption and constitutes the primary component of osteoclast activating factor (40). In the present study, it was found that IL-1β and TNF-α have a correlation with the volume of periapical bone loss, where IL-1β demonstrates a moderate correlation and TNF-α shows a weak one. Furthermore, these findings are consistent with those of Ataonlu et al. (2002) (34), who found that IL-1β is likely to be of greater importance in periapical bone loss compared to TNF-α.

Thus, the results of the present study confirm the correlation of IL-1β and TNF-α with the volume of periapical bone destruction, directly influencing this process.

Ackownedgments

The authors deny any conflicts of interest related to this study.

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3 CONSIDERAÇÕES GERAIS

O combate à infecção endodôntica nos canais radiculares representa um desafio em Endodontia, onde a MIC pode ter um papel substancial durante o tratamento endodôntico. Desta forma, o artigo 1 exposto anteriormente, apresenta a NAC, uma substância química com diversas características promissoras e desejáveis para uma MIC, como a sua atividade antibacteriana (Choi et al., 2018; Martinho et al., 2022; Moon et al., 2016; Olofsson; Hermansson; Elwing, 2003; Palaniswamy et al., 2016; Pérez-Giraldo et al., 1997; Quah et al., 2012; Calefi et al., 2022; Ulusoy et al., 2016; Zhao; Liu, 2010), e a sua capacidade de desorganizar comunidades bacterianas em biofilmes (Choi et al., 2018; Pérez-Giraldo et al., 1997; Quah et al., 2012) o que pode torná-la uma alternativa, além do $Ca(OH)_2$.

Este estudo utilizou a *checkerboard DNA-DNA hybridization* para identificar bactérias em infecções endodônticas primárias em dentes unirradiculares. As amostras iniciais (S1) apresentaram uma média de 35 espécies por canal radicular, confirmando a natureza polimicrobiana dessas infecções (Cavalli et al., 2017; Gomes et al., 2004; Nair, 2004; Siqueira et al., 2004). A espécie bacteriana Gram- negativa mais prevalente foi a *Prevotella melaninogenica*, enquanto *Streptococcus sanguinis* foi a mais prevalente entre as Gram-positivas. Estudos clínicos anteriores relataram uma média menor de espécies bacterianas iniciais por canal radicular (Cavalli et al., 2017; Ferreira et al., 2015; Machado et al., 2020). Além disso, a composição bacteriana após a abertura coronária e antes do preparo biomecânico não coincidiu completamente entre estudos anteriores e o presente estudo (Cavalli et al., 2017; Ferreira et al., 2015; Machado et al., 2020).

Após o preparo biomecânico, houve uma redução significativa no número de espécies bacterianas, mas a composição não mudou significativamente. A etapa MIC resultou em um aumento no número de espécies bacterianas após a preparo biomecânico, exceto para o grupo de medicação com $Ca(OH)_2$ + NAC. *Fusobacterium nucleatum* foi a bactéria mais prevalente após a MIC por 14 dias, especialmente nos grupos de medicação com $Ca(OH)_2$ + solução salina e $Ca(OH)_2$ + NAC. O preparao biomecânico associado a MIC se mostraram cruciais para reduzir a quantidade de endotoxinas.

Além disso, certas espécies bacterianas e níveis mais altos de endotoxinas

estão diretamente relacionados aos sinais e sintomas clínicos (Cavalli et al., 2017; Jacinto et al., 2005). No entanto, devido ao tamanho amostral limitado, não foi possível estabelecer essa relação estatisticamente. Pacientes do grupo NAC + CHX relataram menos dor pós-tratamento, sugerindo melhor eficácia clínica.

A partir dos resultados expostos no artigo 1, foi possível observar que, apesar do baixo espaço amostral, os grupos de MIC associadas à NAC ($Ca(OH)_2$ + NAC e NAC + CHX) apresentaram resultados semelhantes aos do grupo controle ($Ca(OH)_2$ + soro fisiológico) em relação a redução microbiana e de seus subprodutos. Ademais, os sinais e sintomas clínicos dos grupos de medicação associados à NAC foram semelhantes ao do grupo controle ($Ca(OH)_2$ + soro fisiológico). Logo, a NAC se mostra uma alternativa para o $Ca(OH)_2$, o qual é uma MIC consagrada e utilizada há mais de um século em Endodontia (Siqueira; Lopes, 1999).

Há uma deficiência de estudos *in vitro* e *in vivo* que investiguem os efeitos da NAC contra patógenos endodônticos e seus subprodutos, logo, sugere-se que mais pesquisas com espaços amostrais maiores e acompanhamento de longo prazo sejam realizadas para validar os achados do presente estudo e explorar as amplas aplicações clínicas da NAC em Endodontia.

Além disso, no artigo 2 exposto anteriormente, foi analisada a correlação existente entre o volume da destruição óssea periapical e a quantidade de citocinas inflamatórias (IL-1β, IL-6, IL-17a e TNF-α), em casos de infecção endodôntica primária em dentes unirradiculares.

Neste estudo clínico, foi encontrada uma correlação moderada e leve respectivamente entre a quantidade de IL-1β e de TNF-α presente no fluido periapical e o volume de destruição óssea periapical. Esses achados estão alinhados com os resultados obtidos por Popovska et al. (2017), que também relataram uma correlação moderada entre o tamanho das lesões periapicais e a quantidade de IL-1β. Além disso, observou-se que a IL-1β está presente em lesões periapicais crônicas durante estágios de exacerbação aguda e em granulomas nos estágios iniciais de desenvolvimento. Adicionalmente, em um estudo clínico realizado por Bakhsh et al. (2022), foram observados níveis mais altos de IL-1β e IL-6 em lesões periapicais maiores. Em comparação com o presente estudo, esses achados são consistentes com IL-1β, mas não com IL-6, que não mostrou

correlação com o volume de destruição óssea periapical. Em outro estudo clínico realizado por Ataonlu et al. (2002), os níveis de IL-1β e TNF-α em exsudatos periapicais foram analisados, e níveis mais altos de IL-1β foram encontrados em comparação com TNF-α, e maiores quantidades de IL-1β foram associadas a áreas maiores de radioluscência periapical, bem como no presente estudo, onde a correlação com a destruição óssea periapical da IL-1β foi maior em relação ao TNF-α.

No entanto, as citocinas inflamatórias IL-6 e IL-17a não apresentaram correlação. No que concerne a IL-6, os resultados estão alinhados com os achados de Hofbauer et al. (1999), onde o TNF-α e IL-1β estimulam os níveis estáveis de mRNA do ligante da osteoprotegerina, um fator crucial para induzir a osteoclastogênese em várias células da linhagem osteoblástica humana, enquanto IL-6 não demonstrou esse efeito. No entanto, como sugerido por Azuma et al. (2014), durante a formação de lesões periapicais, há um aumento local de IL-6 em resposta à infecção, o que pode induzir a formação de novos osteoclastos a partir de células progenitoras hematopoiéticas, bem como ativar osteoclastos pré-existentes. Isso poderia teoricamente estabelecer uma relação direta entre IL-6 e o volume de lesões periapicais. No entanto, esses achados não se alinham com os resultados deste estudo clínico. Além disso, em um estudo clínico conduzido por Martinho et al. (2012), o tamanho de lesões periapicais observadas radiograficamente correlacionou-se com TNF-α e IL-6, mas não com IL-1β. Embora os resultados para TNF-α entrem em acordo com este estudo, os resultados para IL-6 e IL-1β não encontraram suporte.

De acordo com os achados do presente estudo, IL-17a não apresentou correlação com o volume da destruição óssea periapical. Além disso, entre as citocinas estudadas nesta pesquisa, IL-17a exibiu a menor expressão. Consistente com esses achados, Ajuz et al. (2014) não encontraram correlação entre o volume de lesões periapicais e a IL-17a. Há uma falta de estudos que investiguem a atuação da IL-17a na periodontite apical, destacando a necessidade de mais pesquisas.

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ANEXO A – Certificado do Comitê de Ética

INSTITUTO DE CIÊNCIA E TECNOLOGIA - CAMPUS DE SÃO JOSÉ DOS CAMPOS - UNESP

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: AVALIAÇÃO CLÍNICA DE MEDICAÇÕES INTRACANAL SOBRE MEDIADORES LIPÍDICOS, CITOCINAS INFLAMATÓRIAS E AÇÃO SOBRE BACTÉRIAS E SEUS PRODUTOS EM INFECÇÕES ENDODÔNTICAS DE PACIENTES SAUDÁVEIS E PACIENTES QUE RECEBERAM RADIOTERAPIA DE CABEÇA E PESCOÇO

Pesquisador: Marcia Carneiro Valera Garakis

Área Temática:

Versão: 3

CAAE: 62631122.0.0000.0077

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

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 5.828.002

Apresentação do Projeto:

Este estudo randomizado irá avaliar em dentes com periodontite apical a atividadeantimicrobiana e o efeito sobre produtos bacterianos de protocolos
de tratamento utilizandomedicações intracanal com hidróxido de cálcio [Ca(OH)2], N-acetil cisteína (NAC) e Clorexidina(CHX) e suas associações.
Será subdividido em 2 subprojetos. Subprojeto 1: Avaliará o efeito demedicações intracanal (MIC): Ca(OH)2]+soro, Ca(OH)2+NAC, Ca(OH)2+CHX e
CHX+NAC, nadesinfecção e redução de endotoxinas e LTA do canal radicular em dentes com infecção endodônticaprimária (IEP) e periodontite
apical (PA), bem como seu efeito sobre o perfil inflamatório através daquantificação dos mediadores lipídicos, citocinas inflamatórias e matriz de
metaloproteinases e suaação no processo de reparo ósseo através da expressão de marcadores ósseos. Os pacientes serãosubmetidos à exame
tomográfico (TCFC) para avaliar a volumetria da destruição óssea periapicalutilizando o software Nemotec® e serão avaliados os sinais e sintomas
clínicos presentes antes,durante e após o atendimento. Serão selcecionados dentes unirradiculares com IEP e PA que terãoseus canais preparados

Endereço: Av.Engº Francisco José Longo 777 - Ramal 9028
Bairro: Jardim São Dimas **CEP:** 12.245-000
UF: SP **Município:** SAO JOSE DOS CAMPOS
Telefone: (12)3947-9078 **Fax:** (12)3947-9000 **E-mail:** ceph.ict@unesp.br

utilizando instrumento de lima única reciprocante e irrigação com hipocloritode sódio 2,5%, seguidos das MICs. Serão feitas amostras do conteúdo
dos canais radiculares após aabertura coronária (S1), após o preparo dos canais (S2) e após 15 dias do uso das MICs a fim deavaliar a carga (por
cultura - UFC/mL) e perfil microbiológico (por Cherkerboard DNA-DNAhibridization), níveis de LPS (teste de Lisado de Amebócitos de Limulus – LAL)
e LTA (por ELISA).Serão feitas coletas do fluido periapical após o preparo dos canais (SF1) e após 15 dias da MICs(SF2) para quantificação
mediadores lipídicos (RvD2 e MaR1), inflamatórios (IL-1, IL-6, IL-10 e IL17, MMP-8 e MMP-13) e marcadores ósseos (RANKL, OPG e esclerotina)
que serão realizadosatravés do ensaio imunoenzimático – ELISA e multiplex, respctivamente. Subprojeto 2: Avaliará oefeito de MICs Ca(OH)2+soro,
Ca(OH)2+CHX e Ca(OH)2+NAC na desinfecção e redução deendotoxinas e LTA do canal radicular em dentes com infecção endodôntica primária e
periodontiteapical, em paciente submetidos a radioterapia (RT) de cabeça e pescoço.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar o efeito de medicações à base de hidróxido de cálcio P.A.: [Ca(OH)2]+soro; Ca(OH)2+Nacetilcisteina (NAC); Ca(OH)2 associado à
clorexidina gel 2% (CHX) e CHX+NAC na desinfecção e redução de endotoxinas e LTA do canal radicular em dentes com infecção endodôntica
primária e periodontite apical, bem como seu efeito sobre o perfil inflamatório através da quantificação dos mediadores lipídicos, citocinas
inflamatórias e matriz de metaloproteinases e sua ação no processo dereparo ósseo através da expressão de marcadores ósseos. Avaliar o efeito
das medicações Ca(OH)2+soro; Ca(OH)2+CHX e Ca(OH)2+NAC na desinfecção eredução de endotoxinas e LTA do canal radicular em dentes com
infecção endodôntica primária eperiodontite apical, em paciente submetidos a RT de cabeça e pescoço.

Objetivo Secundário:

•Investigar o status inicial e monitorar a carga e perfil microbiano nos dentes com necrose pulpar epresença de lesão periapical antes do tratamento,

após preparo biomecânico com instrumentoreciprocante e irrigação com NaOCl 2,5%, e após o uso de diferentes medicações intracanais
(MIC):Ca(OH)2+soro, Ca(OH)2+NAC, Ca(OH)2+CHX e CHX+NAC;• Avaliar o efeito do preparo biomecânico e das medicações intracanais em
dentes com infecçãoendodôntica primária e lesão periapical na redução de endotoxinas e LTA presentes no interior doscanais radiculares;• Avaliar o
efeito do preparo biomecânico e das diferentes medicações no metabolismo ósseo atravésda expressão dos marcadores ósseos presentes no fluido
periapical: RANKL, OPG e esclerotina;• Avaliar o efeito da terapia endodôntica e das diferentes medicações na inflamação periapical atravésda
expressão dos mediadores lipídicos RvD2 e Maresina 1, das citocinas inflamatórias IL-1, IL-6, IL-10e IL-17 e das Metaloproteinases de Matriz MMP-
8 e MMP-13 presentes no fluido periapical nas infecçõesendodônticas primárias;• Avaliar o volume da lesão periapical previamente ao tratamento
endodôntico em dentes com necrosepulpar através de imagens de tomografia computadorizada de feixe cônico (TCFC), correlacionandoesse
volume com os mediadores inflamatórios e perfil microbiológico;• Relacionar carga (UFC/mL) e perfil (Cherkerboard) microbiano, os níveis de
endotoxinas (EU/mL) e deácido lipoteicóico (LTA)/mL) com os sinais e sintomas clínicos e volume da lesão periapical (mm³) através douso de
TCFC.•Avaliar o status microbiano inicial do interior dos canais radiculares previamente ao tratamentoendodôntico de dentes com necrose pulpar e
PA em pacientes que receberam RT de cabeça e pescoçoe comparar com aqueles que não receberam RT;• Avaliar o volume da PA previamente ao
tratamento endodôntico em dentes com infecção endodônticaprimária em pacientes que receberam RT de cabeça e pescoço através de imagens de
TCFC;• Avaliar a atividade antimicrobiana do preparo biomecânico com instrumento único reciprocante esolução de NaOCl 2,5% e das MICs:
Ca(OH)2+soro, Ca(OH)2+CHX e CHX+NAC em dentes cominfecção endodôntica primária e lesão periapical de pacientes submetidos a RT de
cabeça e pescoço;• Avaliar o efeito do preparo biomecânico e das MICs Ca(OH)2+soro, Ca(OH)2+CHX e CHX+NAC naredução de endotoxinas e
LTA presentes no interior dos canais radiculares de dentes com infecçãoendodôntica primária e

lesão periapical em pacientes submetidos a
radioterapia de cabeça e pescoço.• Relacionar carga (UFC/mL) e perfil (Cherkerboard) microbiano, os níveis de endotoxinas (EU/mL) e deácido
lipoteicóico (LTA)/mL) com os sinais e sintomas clínicos e volume da lesão periapical (mm³) através douso de TCFC;

Avaliação dos Riscos e Benefícios:

Riscos:

Neste estudo, os pacientes serão submetidos a um tratamento endodôntico convencional, logo, alguns de seus riscos estão nas intercorrências que
podem ocorrer durante a intervenção clínica, dentre elas, podemos citar: (i) desconforto após o tratamento, que pode durar algumas horas ou dias,
sendo necessário, as vezes, o uso de medicamentos, que serão prescritos oportunamente; (ii) fratura de instrumento no interior do conduto radicular,
onde pode-se tentar remover o fragmento clinicamente via canal, e se não for possível, o fragmento pode ser ultrapassado, e a obturação será feita
envolvendo o instrumento. Caso ocorra este tipo de situação clínica, o caso será devidamente proservado, e caso haja necessidade, pode-se optar
por uma cirurgia paraendodôntica; (iii) perfuração da raiz, onde ocorre uma comunicação do meio interno do canal radicular com os tecidos
perirradiculares que envolvem a porção radicular do dente. Ela poderá ocorrer por meio de brocas ou limas, que são utilizadas durante o acesso ao
canal radicular e no preparo biomecânico, respectivamente. Para evitar a perfuração, será feita a radiografia inicial e tomografia computadorizada,
com o intuito de realizar um estudo pré-operatório das características do trajeto do canal radicular, e além disso, caso ocorra uma eventual
perfuração, a obliteração da mesma será feita através de cimentos regeneradores à base de agregado de trióxido mineral (MTA) que será aplicado
com o auxílio de microscopia; e, (iv) o extravasamento da solução irrigadora, neste estudo, será utilizado o hipoclorito de sódio, que é um solvente
de matéria orgânica que pode ter efeitos extremamente irritantes para os tecidos. Caso ocorra extravasamento de hipoclorito de sódio, será
receitado para o paciente analgésico, anti-inflamatório e antibiótico, e se for necessário, o

paciente será encaminhado para acompanhamento
médico. Além das intercorrências que podem ocorrer durante a intervenção, outro risco existente no tratamento está na exposição do paciente aos
raios x, que são radiações ionizantes, que ocorrem durante as tomadas radiográficas feitas no momento da endodontia. No entanto, com o objetivo
de minimizar as chances de repetição deste exame e a consequente exposição aos raios x, os pacientes serão adequadamente protegidos, e a
equipe utilizará técnica radiográfica com posicionador periapical e sensor digital.
Ademais, não serão realizadas outras intervenções ou modificações intencionais nas variáveis fisiológicas, psicológicas ou sociais dos indivíduos
que participarão deste estudo.
A longo prazo, temos o risco do insucesso do tratamento endodôntico realizado nesta pesquisa. Em busca de minimizar as chances de falhas no
tratamento, será feita a radiografia e tomografia pré-operatórias, para estudo minucioso do caso e todos os princípios técnicos e biológicos serão
respeitados. Todos os pacientes que participarão do estudo serão submetidos, após 18 meses transcorridos do término de seu tratamento, à uma
proservação clínica e posterior tomografia computadorizada para análise da evolução da volumetria das lesões periapicais.
Por fim, esta pesquisa envolve o risco de quebra de confidencialidade (algum dado que possa identificar o voluntário a ser exposto publicamente).
para minimizar esse risco, nenhum dado que possa identificar o candidato como nome, codinome, iniciais, registros individuais, informações postais,
números de telefones, endereços eletrônicos, fotografias, figuras, caracteristicas morfológicas (partes do corpo), entre outros, serão utilizadas sem
sua autorização prévia. E, as radiografias que venham a ser utilizadas, estarão devidamente cuidadas para não identificar o paciente.
Benefícios:
Durante o tratamento endodôntico de dentes unirradiculares com periodontite apical de pacientes saudáveis e de pacientes submetidos a
radioterapia de cabeça e pescoço, será realizado um protocolo de atendimento que lança mão de diversos recursos técnicos e biológicos que visam
o sucesso da endodontia, como o exame de tomografia computadorizada, que permite uma visão

tridimensional do canal radicular e da lesão
periapical; a instrumentação mecanizada com limas reciprocantes Reciproc® R40
(VDW, Munique, Alemanha); a utilização de localizador foraminal para uma adequada odontometria; e a medicações intracanal com vistas à redução
de microrganismos e seus subprodutos. E, se porventura o caso apresentar complexidade, o operador poderá fazer uso de microscopia durante o
tratamento endodôntico, outra magnificação que tem o potencial de auxiliar no alcance do sucesso do tratamento. Logo, o paciente será submetido a
um tratamento que utiliza recursos de alta qualidade, que possuem grande efeito na desinfecção dos condutos radiculares

Comentários e Considerações sobre a Pesquisa:

A pesquisadora encaminha a presente emenda, justificando que a pesquisa não é um estudo multicêntrico e será toda realizada na Instituição Instituto de Ciência e Tecnologia-Campus de São José dos Campos-UNESP.

Considerações sobre os Termos de apresentação obrigatória:

Vide conclusões ou pendência e lista de inadequações.

Recomendações:

Vide conclusões ou pendência e lista de inadequações.

Conclusões ou Pendências e Lista de Inadequações:

A emenda atende a solicitação da pesquisadora.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_2036238_E1.pdf	30/11/2022 14:57:22		Aceito
Parecer Anterior	Formulario_Pendencias_CEP.pdf	23/09/2022 15:41:45	Marcia Carneiro Valera	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termo_de_Consentimento.pdf	21/09/2022 22:47:32	Marcia Carneiro Valera	Aceito
Folha de Rosto	folha_de_rosto.pdf	04/08/2022	Marcia Carneiro	Aceito

INSTITUTO DE CIÊNCIA E TECNOLOGIA - CAMPUS DE SÃO JOSÉ DOS CAMPOS - UNESP

Continuação do Parecer: 5.828.002

Folha de Rosto	folha_de_rosto.pdf	18:20:12	Valera	Aceito
Projeto Detalhado / Brochura Investigador	Projeto.pdf	02/08/2022 23:26:17	Marcia Carneiro Valera	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

SAO JOSE DOS CAMPOS, 19 de Dezembro de 2022

Assinado por:
Denise Nicodemo
(Coordenador(a))

Endereço: Av.Engº Francisco José Longo 777 - Ramal 9028
Bairro: Jardim São Dimas **CEP:** 12.245-000
UF: SP **Município:** SAO JOSE DOS CAMPOS
Telefone: (12)3947-9078 **Fax:** (12)3947-9000 **E-mail:** ceph.ict@unesp.br

ANEXO B — Termo de Consentimento Livre e Esclarecido

Termo de Consentimento Livre e Esclarecido

Caro(a) Senhor(a),

Você está sendo convidado(a) para participar, como voluntário(a), em uma pesquisa científica. Caso você não queira participar, não será necessário justificar o motivo, visto que é seu direito a escolha em participar deste estudo, podendo realizar o tratamento sem nenhuma alteração.

Para confirmar sua participação você precisará ler todo este documento, na qual estão contidas as principais informações sobre o estudo, objetivos, riscos e benefícios, dentre outras informações.

Este Termo de Consentimento Livre Esclarecido (TCLE) se refere ao projeto de pesquisa **"AVALIAÇÃO CLÍNICA DE MEDICAÇÕES INTRACANAL SOBRE MEDIADORES LIPÍDICOS, CITOCINAS INFLAMATÓRIAS E AÇÃO SOBRE BACTÉRIAS E SEUS PRODUTOS EM INFECÇÕES ENDODÔNTICAS DE PACIENTES SAUDÁVEIS E PACIENTES QUE RECEBERAM RADIOTERAPIA DE CABEÇA E PESCOÇO"**, cujo objetivo é tratar uma doença dentária aplicando técnicas já utilizadas e pretendemos avaliar a resposta do paciente a diferentes medicações comparando os dados obtidos de pacientes saudáveis e pacientes submetidos à radioterapia de cabeça e pescoço.

Você não será remunerado, visto que sua participação nesta pesquisa é de caráter voluntário. Os pesquisadores garantem e se comprometem com o sigilo e a confidencialidade de todas as informações fornecidas por você para este estudo.

A presente pesquisa apresenta como responsável Márcia Carneiro Valera Garakis, professora titular da Disciplina de Endodontia da Faculdade de Odontologia de São José dos Campos - UNESP, portador 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.

O objetivo deste estudo será avaliar o efeito do tratamento endodôntico (tratamento de canal) através do uso de diferentes tipos de medicações que serão colocadas no interior do canal radicular, na eliminação de bactérias e seus produtos dos canais e a atuação dessas medicações no processo inflamatório presente nos tecidos ao redor dos dentes. Serão selecionados 105 pacientes, com necessidade de tratamento endodôntico que serão divididos em 7 grupos (n=15). Realizaremos coletas durante o procedimento, feitas com cones de papel absorvente, do líquido no interior do canal do dente a ser tratado e, em seguida, em laboratório, o material que foi coletado será submetido a análise.

O paciente como beneficiário, receberá tratamento endodôntico, com técnicas avançadas, seguindo protocolos consagrados que contribuirão para o sucesso do caso. O projeto proposto poderá acarretar em riscos mínimos ao paciente por se tratar de um procedimento invasivo; podem ocorrer imprevistos durante o atendimento em função do próprio tratamento. No caso de algum

imprevisto, o paciente será amparado e receberá todos os cuidados necessários para controlar quaisquer eventualidades. Os medicamentos a serem utilizados possuem comprovação científica de ausência de efeitos tóxicos para o organismo. Além disso, todo o tratamento de canal será realizado dentro das normas de biossegurança e serão realizados por profissionais altamente capacitados; os resultados obtidos trarão benefícios para outras pessoas uma vez que a pesquisa irá gerar avanços científicos com melhoras no tratamento de canal realizado na atualidade. Serão necessários 2 atendimentos (3 horas cada atendimento) com um período de 14 dias de intervalo entre cada sessão até a conclusão do tratamento. A responsável pelo tratamento é a Cirurgiã Dentista e aluna de Pós Graduação Mariana Muniz Toledo Orlando, (celular: (12)982604237 email:mariana.muniz@unesp.br) e em caso de dúvida ou necessidade de intervenção, deve-se entrar em contato com ela. Os pacientes serão chamados para acompanhamento radiográfico após 1 ano do tratamento finalizado. Além disso, o protocolo de conduta adotado é o mesmo já utilizado na prática clínica odontológica desta faculdade.

Se houver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética (CEP) do Instituto de Ciências e Tecnologia de São José dos Campos-ICT/ UNESP, situada na Av. Engº Francisco José Longo, 777 – CEP12.245-000, em São José dos Campos – SP, fone (12) 3947-9000. Informo que será garantida a liberdade da retirada do consentimento a qualquer momento e assim deixar de participar do estudo.

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 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