MARCELA DE FARIA MOURA

EFEITO DO TRATAMENTO PERIODONTAL NÃO CIRÚRGICO EM INDIVÍDUOS COM ARTRITE REUMATÓIDE E PERIODONTITE: *ASPECTOS EPIDEMIOLÓGICOS, CLÍNICOS, MICROBIOLÓGICOS E BIOMARCADORES*

FACULDADE DE ODONTOLOGIA
UNIVERSIDADE FEDERAL DE MINAS GERAIS
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Tese apresentada ao Colegiado de Pós-Graduação da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial para a obtenção do título de Doutor em Odontologia.
Área de concentração: Periodontia.

Orientador: Prof. Dr. Fernando de Oliveira Costa.

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COLEGIADO DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

ASSOCIAÇÃO ENTRE ARTRITE REUMATÓIDE E PERIODONTITE: ASPECTOS EPIDEMIOLÓGICOS, CLÍNICOS, MICROBIOLÓGICOS E IMUNOLÓGICOS

MARCELA DE FARIA MOURA

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Odontologia, como requisito para obtenção do grau de Doutor, área de concentração Periodontia.

Aprovada em 11 de dezembro de 2020, pela banca constituída pelos membros:

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Profa. Isabela Almeida Pordeus
Coordenadora
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Amiga Lu, Ana, Paulo Víctor: preciso desestressar; Má e Cláudio: posso ficar uns dias aí na casa de vocês, eu e a Nina queremos mudar para aí. Mamãe: acende uma vela; pede tia Maria, tia Solange e tia Nilza para fazerem uma novena para mim. Tia Rose e tia Celina: me acompanha em um vinho? Graças a Deus está dando tudo certo!

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Laboratório de patologia, março de 2014, análises do mestrado sendo realizadas. @Tarcilia pergunta: @Marcela, você já fez seu projeto para a seleção do doutorado? @Fernando, o que você acha? Escreve e manda para mim.

Restaurante Saatori, nos dias da seleção: @ Ma, @Claudio, @Lê e @Pedro vamos jantar, rezar e torcer e torcer para dar certo.

Hospital Risoleta Neves, início da pesquisa do doutorado- projeto piloto. Mudança de projeto após 2 anos de doutorado, o melhor a ser feito. Novamente no laborário: @Tarcília, eu pensei em trabalhar com Artrite e Periodontite, o que você acha? Marca uma reunião.

Anseio de descobrir coisas novas, competência em tudo que faz, compromissada. Esta conexão com a @Tarcília foi fundamental para a construção do nosso trabalho de mestrado e doutorado.

O contato com a @Tarcília me trouxe também um casamento de quase 6 anos com um freezer - 80 graus que necessitava de cuidados diários. Os 2 anos iniciais foram de lua de mel, os 2 subsequentes caíram em uma rotina que funcionava bem, mas nos dois últimos anos, relação desgastada. Minhas amostras do doutorado eu preferi manter no freezer da @Tarcíllia, mais seguro...rs.

5° e 6° ano na UFMG, Hospital das clínicas (HC), @Gilda abriu as portas da reumatologia para mais uma parceria com a Faculdade de Odontologia. Dias que alimentei minha fé, as dificuldades eram inúmeras. Com superação, resiliência e ajuda dos funcionários, técnicos e enfermeiros da reumatologia, a pesquisa começou a caminhar.

Pacientes de um ambulatório, muito desgastados, após infinitas pesquisas da odonto, farmácia, medicina, fisioterapia. Como convencê-los de participar de mais uma pesquisa? Você ainda tem dentes? Vamos tratar seus problemas de gengiva? Será um ensaio clínico com diversas consultas...

Férias e greve na UFMG. Eu e @Bianca passamos os 4 meses tratando pacientes, embora a faculdade estivesse fechada. Quando as aulas voltaram, @Mauro permitiu que nós continuássemos a tratar nossos pacientes, mesmo

não sendo permitido alunos de Pós-graduação atender sem o acompanhamento de um professor responsável.

Uma antiga parceria do @ Fernando com @ José Roberto Cortelii e @ Sheila Cortelli da Universidade de Taubaté, me proporcionou momentos de aprendizado no laboratório da UNITAU, além de resultados microbiológicos fundamentais para nosso estudo.

@Alcione (PUC) e @Luís reservaram um tempo para acrescentar no nosso trabalho e participar da banca da qualificação; além de ajudarem nas pesquisas e nos artigos.

Oi @ Fernando, tudo bem? Sei que você já ouviu todas a desculpas possíveis na vida dos seus orientandos. Porém, pandemia do Corona Vírus, esta, com certeza é a primeira vez! Tudo tem dois lados na vida, com a Covid-19 e o lock down, @Fernando rapidamente me ajudou com dois artigos que tínhamos urgência para finalizar. Meu prazo para a defesa era até final de julho 2020.

A pandemia estava no seu auge na época que seria a minha defesa. Os kits para as análises laboratoriais que o Colegiado (@Isabela Pordeus) havia comprado para a gente, não chegava. Voltando para Belo Horizonte, depois de 6 meses na roça, @ Tarcília e @Sicília realizaram as análises laboratoriais após a chegada dos Kits.

Trabalho, projetos, aulas, vida pessoal e viagens. A cada dia de convivência com o @Fernando e @Luis, sempre tentei ser honesta e verdadeira nas nossas relações. Acho que para nós, a confiança e o respeito regeram os anos vividos juntos.

@ Fernando tem um jeito único, faz a gente passar muitos apertos, mas é generoso quando sente que precisamos realmente da sua ajuda. Foi considerado um dos pesquisadores mais influentes do mundo e, certamente, sua influência na minha vida será para sempre valorizada.

@Flavinho me dá um curso para aprender tirar sangue? Claro, será em Itabira. @Fabi como vou conseguir seguir com esta pesquisa? Vem tomar um café aqui em casa hoje. @Paulinha vamos programar o cardápio e a organização do fim de semana na roça? @Toninho não vai dar tempo de programar tudo, providencia algumas coisas para mim, pede para arrumar meu quarto, coloca um vinho para gelar! @ D. Marlene – mamãe- o que vamos

comer hoje, estamos de dieta! Já acendeu a vela para Nossa Senhora Aparecida e de @Fátima?

@ Divino Espírito Santo e meu anjo da guarda, foram eles que me acordaram de madrugada para ir para o hospital captar pacientes, me deram força para tentar infinitos contatos para marcar tratamento e retorno dos pacientes, me deram sabedoria para conseguir adequar às novas regras e exigências da nova coordenação da Pós-graduação, após o programa receber a melhor nota de qualidade do país.

@Cláudio, meu computador travou, me ajuda aqui com esta planilha, vamos montar mais um banco de dados. @ Ma, eu preciso de ajuda, o que acha que eu falo na reunião? Somente vocês realmente sabem como foi o dia a dia destes anos... Ansiedade com provas, com cada nova etapa da pesquisa que se iniciava. Preocupações com minhas amostras e com a de todos os colegas que estavam no freezer, com os prazos que precisavam ser cumpridos. @Cláudio sempre generoso em ouvir meus infinitos desabafos e disponível para qualquer coisa que pudesse ajudar.

@Ma é simplesmente a pessoa mais importante em toda a minha formação até aqui. Foi enérgica enquanto eu fazia cursinho, era telefonista e precisava passar no vestibular em Universidade Federal. Na faculdade, tentava me influenciar para gostar de uma especialidade que pudesse encaixar-se na dela. Parceria no trabalho, apoio na especialização de implante, incentivo para uma outra especialização de Perio. Nunca imaginamos que seria tão longo e desgastante um caminho de mestrado e doutorado. Ela vivenciou ao meu lado, cada sentimento vivido. Deu força, incentivo, foi compreensiva, me ouviu e deu conselhos. E claro, sempre comemoramos muito cada artigo aceito, cada bom momento e todas as pequenas vitórias até a conclusão desta Tese de doutorado. Obrigada a todos que dividiram comigo a minha formação para doutora!

RESUMO

Objetivos: Evidências atuais reconhece que a periodontite (PE) e a artrite reumatóide (AR) compartilham comuns fatores de risco e alguns caminhos fisiopatológicos semelhantes, como inflamação crônica induzida por citocinas pró-inflamatórias e destruição de tecidos conjuntivos e ósseos. Assim, esta tese apresenta quatro estudos da possível associação entre PE e AR com os seguintes objetivos: avaliar a condição periodontal, gravidade e extensão da periodontite e aspectos clínicos e epidemiológicos da sua associação com a AR. Investigar a influência do tratamento periodontal não cirúrgico (TPNC) sobre os parâmetros clínicos periodontais, níveis bacterianos de *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T dentícola* e a atividade da AR (DAS28). Adicionalmente quantificar níveis sanguíneos e salivares dos biomarcadores RANKL, OPG, RANKL / OPG, Survivina, e anticorpos antipeptídeos citrulinados cíclicos (ACPAs) e anti-carbamiladas (Anti-CarP).
Métodos: A amostra do estudo foi composta por um total de 471 indivíduos divididos aleatoriamente em grupos, de acordo com o objetivo de cada estudo. O ensaio clínico realizou as seguintes avaliações no início do estudo (T1) e 45 dias (T2) após TPNC: exames periodontais de boca completa, análise microbiológica *por meio de qPCR em tempo real*, avaliações do Disease Activity Score (DAS-28). Em adição, a quantificação de biomarcadores e anticorpos Anti-Carp e ACPAs realizada por meio de ELISA no soro/saliva.
Resultados: O presente estudo reportou uma alta prevalência de PE em indivíduos com AR e uma importante associação entre ocorrência, gravidade e extensão da PE associadas à AR e ao tabagismo. O tratamento periodontal não cirúrgico foi eficaz em melhorar a condição cliníca periodontal, reduziu a presença dos patógenos periodontais avaliados e melhorou o quadro clínico da AR (redução dos escores de DAS-28). Adicionalmente, reduziu os níveis de survivina, RANKL e concentração de ACPAs e Anti-CarP.
Conclusão: Nossos achados corroboram a robustez das evidências científicas da associação entre periodontite e AR. Assim, o controle da infecção periodontal em pacientes com AR e PE pode ser uma importante ferramenta terapêutica no controle da AR.
Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/).

Palavras-chave: Artrite reumatoide. Periodontite. Estudo caso-controle. Bactérias. Marcadores biológicos. Citocinas. Ensaios clínicos. Terapia periodontal não cirúrgica. Fatores de risco.

ABSTRACT

Effect of non-surgical periodontal treatment in individuals with rheumatoid arthritis and periodontitis: epidemiological, clinical, microbiological and biomarker aspects

Objectives: Current evidence recognizes that periodontitis (PE) and rheumatoid arthritis (RA) share common risk factors and some similar pathophysiological pathways, such as chronic inflammation induced by pro-inflammatory cytokines and destruction of connective and bone tissues. Thus, this thesis presents four studies of the possible association between PE and RA with the following objectives: to assess the periodontal condition, severity and extent of periodontitis and the clinical and epidemiological aspects of its association with RA. To investigate the influence of non-surgical periodontal treatment (TPNS) on the periodontal clinical parameters, bacterial levels of A. actinomycetemcomitans, P. gingivalis, T. forsythia, T dentic and the activity of RA (DAS28). In addition to quantify blood and salivary levels of the biomarkers RANKL, OPG, RANKL / OPG, Survivina, and cyclic citrullinated anti-peptide antibodies (ACPAs) and anti-carbamylates (Anti-CarP).
Methods: The study sample consisted of a total of 471 individuals randomly divided into groups, according to the objective of each study. The clinical trial performed the following assessments at baseline (T1) and 45 days (T2) after TPNC: periodontal examinations of full mouth, microbiological analysis using real-time qPCR, assessments of the Disease Activity Score (DAS-28). In addition, the quantification of biomarkers and Anti-Carp antibodies and ACPAs performed by ELISA in serum / saliva.
Results: The present study reported a high prevalence of PE in individuals with RA and an important association between the occurrence, severity and extent of PE associated with RA and smoking. The non-surgical periodontal treatment was effective in improving the clinical periodontal condition, reduced the presence of the periodontal pathogens evaluated and improved the clinical picture of RA (reduced DAS-28 scores). Additionally, it reduced the levels of survivin, RANKL and concentration of ACPAs and Anti-CarP.
Conclusion: Our findings corroborate the robustness of the scientific evidence on the association between periodontitis and RA. Thus, the control of periodontal infection in patients with RA and PE can be an important therapeutic tool in the control of RA.

Keywords: Rheumatoid arthritis. Periodontitis. Case-control study. Bacteria. Biological markers. Cytokines. Clinical trials. Non-surgical periodontal therapy. Risk factors.

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LISTA DE ABREVIATURAS E SIGLAS

Anti-Carp	Anticorpos Contra Proteínas Carbamiladas
ACPAs	Anticorpos Anti-Proteína/ Peptídeo Citrulinados
PPAD	Anticorpos Anti P. Gingivalis
AR	Artrite Reumatoid
BOP	Bleending On Probing
CAL	Clinical Attachment Level
DAS-28	Disease Activity Scores-28
TNF-α	Fator De Necrose Tumoral-A
FR	Fator Reumatoide
FMD	Full-Mouth Disinfection
IL-1b	Interleucina -1b
IL-6	Interleucina 6
PMNs	Neutrófilos Polimorfonucleares
NIC	Nível De Inserção Clínica
NSPT	Nonsurgical Periodontal Therapy
OPG	Osteoprotegerin
AMPs	Peptídeos Antimicrobiano
PAD	Peptidil Arginina Desiminas
PE	Peridontite / Periodontiti
PD	Probing Depth
PS	Profundidade De Sondage
PCR	Reação Em Cadeia Da Polimerase
NETs	Redes Extracelulares De Neutrófilos
RA	*Reumathoid Arthritis*
SS	Sangramento A Sondagem
TPNC	Teapia Periodontal Não Cirúrgica

SUMÁRIO

1- IDENTIFICAÇÃO E QUALIFICAÇÃO DO PROBLEMA

A periodontite (PE) é uma doença infecto-inflamatória crônica multifatorial, caracterizada por uma progressiva destruição dos tecidos de suporte dos dentes e sua evolução pode levar à perda dos dentes. Este processo é causado por bactérias anaeróbias facultativas, que liberam, principalmente, enzimas proteolíticas com alto potencial de virulência (TONETTI & DYKE, 2013). A doença resulta de uma infecção polimicrobiana complexa, que leva à destruição tecidual em consequência da perturbação da homeostase entre a microbiota subgengival e as defesas imunológicas do hospedeiro (disbiose), em indivíduos suscetíveis (SANZ et al., 2011). Há uma considerável variação individual na resposta do hospedeiro ao biofilme microbiano. Isto ocorre devido a diversos aspectos da resposta inata, inflamatória e imune de cada indivíduo (KINANE et.al., 2011; TONETTI, GREENWELL & KORNMAN, 2018).

Para o diagnóstico da PE, além dos padrões clínicos da doença, biomarcadores têm o potencial de fornecer informações adicionais sobre a patogênese da doença (KINANE et.al., 2011). Dentre esses biomarcadores, numerosas citocinas desempenham papéis-chave na fisiopatologia da PE. As evidências mais fortes são de elevados níveis de interleucina (IL) -1b, fator de necrose tumoral-α (TNF-α), IL-6 e RANKL Kinade et al. (2011) e novos promissores biomarcadores têm sido investigados (LI et al., 2018). Os níveis destes marcadores poderiam influenciar na susceptibilidade, gravidade e desfecho da doença.

A artrite reumatóide (AR) é uma doença auto-imune crônica que provoca uma quebra da auto-tolerância, inflamação crônica e destruição articular debilitante (Gary S. FIRESTEIN, 2003). É caracterizada pela presença do fator reumatoide (FR) e anticorpos anti-proteína/ peptídeo citrulinada (ACPAs) que estão relacionados à atividade da doença (LI et al., 2018). Estudos apontam para uma potencial associação entre a PE e a AR por apresentam caminhos fisiopatológicos semelhantes decorrentes de cascatas inflamatórias crônicas desreguladas, além de fatores ambientais e genéticos

desempenharem um papel crucial, potencializando a destruição tecidual (CULSHAW, MCINNES & LIEW, 2011; KINANE et.al., 2011).

Na presente década, à associação entre PE e doenças inflamatórias imuno-mediadas tem sido reportada de forma exponencial (KINANE et al., 2011; LI et al., 2018; YUCE et al., 2017). Uma recente revisão de literatura, apresenta uma visão geral sobre importantes avanços na compreensão da etiopatogenia da PE, no que diz respeito às evidências crescentes sobre as associações independentes entre PE e diversas condições de saúde. Além da associação com doenças já consolidadas como diabetes e doenças cardiovasculares, apresenta também muitas outras doenças sistêmicas incluindo doenças metabólicas, obesidade, certos cânceres, doenças respiratórias e distúrbios cognitivos e AR (GENCO & SANZ, 2020).

Especificamente, diversos estudos epidemiológicos e clínicos têm reportado que a PE pode ser um fator de risco putativo para a AR ou piorar a condição clínica e sorológica da AR (BELIBASAKIS et al., 2012 CHEAH et al., 2020; COSGAREA et al., 2019; NESSE et al., 2012; REICHERT et al., 2015; SCHMICKLER et al., 2017; YUCE et al., 2017). Essa associação pode ser causada por i) a coexistência de fatores de risco comuns para as duas doenças, como idade, tabagismo e sexo; ii) um comum desequilíbrio imunorregulatório; iii) compartilhamento de fatores de risco genéticos subjacentes; e / ou iv) a possibilidade de que bactérias periodontopatogênicas possam contribuir para a etiologia das doenças reumáticas (YUCE et al., 2017).

Elevados níveis de interleucina IL-1b, IL-6, TNF-α e RANKL também podem influenciar na susceptibilidade, gravidade e desfecho da doença. Os neutrófilos polimorfonucleares (PMNs) também poderiam causar destruição tecidual presente na PE devido à liberação espécies reativas de oxigênio, colagenases e proteases (KINANE, PRESHAW, & LOOS, 2011). Na resposta inflamatória imune do indivíduo com PE, os peptídeos antimicrobianos AMPs (produzidos por neutrófilos e células epiteliais) possuem efeitos inibitórios sobre lipopolissacarídeos (secretados por patógenos orais), citocinas proinflamatórias (produzidas por células T auxiliares e macrófagos), quimiocinas (produzidas por células dendríticas), além de possuir uma atividade antimicrobiana eficaz

contra patógenos orais. Têm sido sugerido que estes patógenos orais, como fontes de repetidas bacteremias sistêmicas transitórias, podem aumentar a suscetibilidade ao diabetes e à AR por meio da disseminação metastática da inflamação (LI et al., 2018).

Embora, AR e DP apresentem diferentes etiologias, os mecanismos patogênicos parecem ser semelhantes e é possível que indivíduos que apresentem tanto a PE quanto a AR possam apresentar uma desregulação da resposta inflamatória (MERCADO et al., 2001). As primeiras células a migrar para os o sítio periodontal inflamatório são os neutrófilos que desempenham um papel essencial na “linha de defesa” do organismo (HIRSCHFELD, 2014). Estas células participam da fagocitose e morte de patógenos por mecanismos que envolvem a degranulação e formação de redes extracelulares de neutrófilos (NETs) (BORREGAARD 2010; MANTOVANI et al., 2011).

NETs são redes fibrosas constituídas por componentes nucleares e por 8 componentes granulares que desempenham função antimicrobiana (BRINKMANN et al., 2004). Essas redes se projetam da membrana de neutrófilos ativados em resposta à infecção e também em resposta ao processo inflamatório estéril (FUCHS et al., 2007). Demonstrou-se que a desregulação na geração e degradação de NETs está envolvida na patogênese de doenças imunomediadas como a AR (LEE et al., 2017). Evidências sugerem que os antígenos citrulinados produzidos na AR são derivados principalmente de NETs (HE et al., 2018).

Corroborando com a possível associação entre a AR e a PE, é importante ressaltar que níveis abundantes de NETs também são encontrados no fluido gengival crevicular de pacientes com PE Vitkov et al. (2010) e que o aumento de NETs na cavidade oral de pacientes com PE pode estar envolvido no desenvolvimento da AR, pelo aumento de peptídeos citrulinados (HE et al., 2018). Entretanto a compreensão do envolvimento de NETs na patogênese dessas duas doenças crônicas inflamatórias e na autoimunidade, e o papel da doença periodontal no desenvolvimento da AR ainda não estão elucidados.

Além destes fatores de similaridade entre a PE e a AR, achados fornecem evidências para uma relação entre a presença de inflamação associada a patógenos periodontais e o desenvolvimento da AR (BARTOLD et al., 2010). Patógenos como *Aggregaticabcter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema dentícola, Prevotella intermedia, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus), E nodatum, E corrodens and Capnocytophaga species (Cs*), são reconhecidos patógenos envolvidos na PE (LI et al., 2018). Particularmente, a *P. gingivalis*, patógeno comum, poderia citrulinar proteínas/peptídeos, induzindo a resposta auto-imune e a susceptibilidade dos indivíduos à AR. Isto poderia ocorrer devido a anticorpos contra proteínas/peptídeos citrulinados constituírem um achado sorológico comum tanto na AR quanto na PE (ROUTSIAS et al., 2011).

Outra modificção pós-traducional contra anticorpos que pode ser encontrada em indivíduos com AR é a carbamilação. A carbamilação é a modificação química de uma lisina em homocitrulina (VERHEUL et al., 2018). A formação de proteínas/peptídeos carbamilados ocorre em um baixo nível em indivíduos saudáveis, mas suas taxas aumentam em várias condições de inflamação como insuficiência renal, doenças cardiovasculares, AR e tabagismo. A carbamilação causa mudanças na função proteica que podem interferir nas funções celulares de uma ampla gama de proteínas e de pequenas moléculas. Isto gera um acúmulo de proteínas/peptídeos carbamilados e interfere nas funções celulares, podendo desencadear desordens sistêmicas ou exacerbar o curso de uma doença (LI et al., 2018; KANEKO et al., 2018).

A carbamilação, com as mudanças das funções proteicas, coloca as proteínas modificadas no alvo da resposta auto-imune levando à formação de anticorpos contra proteínas/peptídeos carbamilados, os anticorpos anti-CarP, em indivíduos susceptíveis (SHI et al., 2014). A carbamilação de proteínas criadas como resultado de inflamação gengival crônica, observada na PE, geraria a produção de anticorpos anti-CarPA, durante a inflamação gengival. Desta forma, poderia existir uma associação entre a produção de anticorpos

anti-CapP à mucosa oral inflamada, em indivíduos com PE (BRIGHT, PROUDMAN, ROSENSTEIN, & BARTOLD, 2015). Além disso, os níveis circulantes de proteínas carbamiladas foram associados à gravidade da PE e influenciados pelo tratamento periodontal em pacientes com AR (KANEKO et al., 2018).

Nos organismos multicelulares, a homeostase é mantida através de um equilíbrio entre a proliferação celular e a morte celular. A morte celular fisiológica ocorre principalmente por apoptose. Evidências recentes sugerem que alterações na sobrevivência celular contribuem para a patogênese de várias doenças, incluindo câncer, infecções virais, doenças autoimunes, distúrbios neurodegenerativos e AIDS (THOMPSON, 1995). A regulação intracelular dos mecanismos de apoptose é realizada pelas proteínas “linfoma de células B 2” (Bcl2) e o fenômeno é basicamente regulado pelo equilíbrio entre que proteínas pró-apoptóticas e inibidoras de apoptose (IAPs).

A Survivina é uma proteína multifuncional que codifica um gene humano (BIRC5) de um inibidor de apoptoste (IAP). Está presente durante o desenvolvimento fetal e é indetectável em tecidos adultos diferenciados terminalmente. Contudo, a Survivina é proeminentemente expressa em linhagens de células alteradas presentes nos cânceres humanos mais comuns de pulmão, cólon, pâncreas, próstata e mama (AMBROSINI et al., 1997).

Níveis mais altos de Survivina foram encontrados em amostras de tecido gengival de indivíduos com PE crônica (LUCAS et.al., 2010). Além disto, foi demonstrado que um importante patógeno periodontal, a *P. gingivalis*, podeira promover a apoptose, desta maneira a apoptose estaria envolvida na doença periodontal, podendo ser regulada por várias moléculas anti e pró-apoptóticas incluindo a Survivina (GEATCH et al., 1999; URNOWEY et al., 2006). Ela seria induzida nos tecidos periodontais por fatores microbianos e do hospedeiro, assim, os mecanismos apoptóticos poderiam estar envolvidos no processo inflamatório associado à destruição do tecido gengival de indivíduos com PE (GAMONAL et al., 2001).

Adicionalmente, um dos mecanismos responsáveis pela remodelação óssea é a apoptose, e sendo suscetíveis à manipulação terapêutica, os osteoclastos são extensivamente empregados para investigar a resposta celular a terapias para o tratamento da perda óssea associada a várias doenças, incluindo PE, osteoporose e osteólise metastática (PENOLAZZI et al., 2008).

A apoptose também foi demonstrada no tecido sinovial de indivíduos com AR com a expressão de níveis mais elevados de Survivina em indivíduos com doença destrutiva mais erosiva. Os anticorpos anti-survivina pode exibir funções protetoras em indivíduos com AR; determinando assim, o curso erosivo da AR (BOKAREWA et al., 2005). Adicionalmente, níveis elevados de Survivina foram associados à tecidos sinoviais de indivíduos com AR ativa, ao tabagismo, à ACPAs e progressão da atividade da AR (DAS28) (DHARMAPATNI et al., 2009; SVENSSON et al., 2014).

A elevação da Survivina na fase pré-clínica da AR e sua associação com ACPAs sugerem a Survivina como um preditor da AR, como um biomarcador de dano articular e de pobre resposta ao tratamento anti-reumático em indivíduos com AR (MANDANA et al., 2020).

A compreensão dos mecanismos moleculares do metabolismo ósseo e da remodelação óssea, permitiu identificar as moléculas RANKL e seu receptor ligante OPG, membros da família do fator de necrose tumoras (TNF), como um dos principais reguladores do desenvolvimento e da função dos osteoclastos. A sinalização RANKL ativa uma variedade das vias de sinalização a necessárias para o desenvolvimento de osteoclastos, além de realizar um *crosstalk* com outras vias de sinalização, ajustando a homeostase óssea tanto na fisiologia normal quanto na doença. Assim, estão envolvidos na fisiologia óssea de várias doenças associadas à perda óssea, como AR, PE, metástases de câncer e osteoporose (LEIBBRANDT & PENNINGER, 2008).

Na PE, foi demonstrado que RANKL é regulado positivamente, enquanto OPG, um protetor do osso, é regulado negativamente em comparação com a saúde periodontal, resultando em uma razão RANKL / OPG

aumentada. Estes marcadores podem ser detectados no tecido gengival e fluidos biológicos, incluindo GCF, saliva e sangue (BELIBASAKIS et al., 2012).

Na AR, a expressão de RANKL ocorre nos fibroblastos sinoviais das articulações e é predominantemente responsável pela formação de osteoclastos, destrutivos ósseos, e erosões durante o processo inflamatório (DANKS et al., 2015). Já a OPG protege a integridade óssea ao diminuir a osteoclastogênese e promove a apoptose dos osteoclastos. O nexo entre a ativação de células T, produção de TNF-α, RANKL e sistema OPG / RANK na RA fornece uma visão sobre o mecanismo dos principais padrões de perda óssea na AR (ROMAS et al., 2002). Após o conhecimento da estrutura molecular chave para o entendimento da fisiologia óssea, a inibição da função RANKL concebeu novas e promissoras estratégias para o tratamento de doenças ósseas, como e prevenir a destruição óssea e danos à cartilagem em indivíduos com osteoporose e AR e a perda óssea associada à PE (BELIBASAKIS et al., 2012; DANKS, et al., 2015; LEIBBRANDT & PENNINGER, 2008).

A concentrações de biomarcadores relevantes na patogênese das doenças tem sido investigada no âmbito oral e sistêmico, por meio de substratos como, saliva e sangue. A saliva apresenta relevância para eventos fisiológicos e patológicos do corpo humano. Devido à sua facilidade de coleta e análise, há interesse recente no uso de saliva no diagnóstico de patologias orais e sistêmicas. Porém não se sabe se é comparável ou homológo o conteúdo da saliva ao soro (sangue); este, amplamente utilizado na triagem de alterações biológicas indicativas do início ou progressão de diversas doenças (BEHFARNIA et al., 2016; BROWNE et al., 2013; LAHDENTAUSTA et al., 2018). Medidas de analitos em saliva e soro, portanto, oferecem um método conveniente para comparar e avaliar o papel dos biomarcadores em compartimentos orais e sistêmicos (BROWNE et al. 2013).

Neste cenário, a presente tese busca contribuir para a compreensão da associação da PE e AR em seus aspectos clínicos e epidemiológicos por meio de um estudo caso-controle, e por três ensaios clínicos controlados

clínicos, abordando aspectos microbiológicos e biomarcadores de interesse entre estas duas doenças.

2- OBJETIVOS

2.1- Estudo 1 - caso-controle: avaliar a condição periodontal, gravidade e extensão da periodontite e aspectos clínicos e epidemiológicos da sua associação com a AR por meio de um estudo caso-controle.

2.2- Estudo 2 - ensaio clínico microbiológico: investigar em indivíduos com AR e PE a influência do tratamento periodontal não cirúrgico (TPNS) sobre a condição clínica periodontal, níveis bacterianos de *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T dentícola* e a atividade da AR por meio de um ensaio clínico controlado randomizado.

2.3- Estudo 3 - ensaio clínico biomarcadores: investigar a influência do TPNS nos parâmetros clínicos periodontais, nos níveis sanguíneos e salivares dos biomarcadores RANKL, OPG, RANKL / OPG e Survivina e a atividade da RA por meio de um ensaio clínico controlado em indivíduos com AR e PE.

2.4- Estudo 4 - ensaio clÍnico anti-CarP e ACPAs: investigar a influência do TPNS no estado clínico periodontal, atividade da AR, carga bacteriana subgengival de *Porphyromonas gingivalis* e concentração de ACPAs e Anti-CarP por meio de um ensaio clínico piloto controlado em indivíduos com AR e PE.

3- HIPÓTESES

3.1- Estudo 1: caso-controle

Indivíduos com AR apreseantam maior frequência, gravidade e extensão de PE que controles saudáveis. Adicinalmente, o tabagismo pode ter um efeito sinérgico nesta associação.

3.2- Estudo 2: ensaio clínico microbiológico

O tratamento periodontal não cirúrgico melhora os parâmetros clínicos periodontais e reduz os níveis dos patógenos *P. gingivalis, T. forsythia, A. actinomycetemcomitans, T. denticola* em indíviduos com AR, melhorando o quadro clínico da AR pela redução do DAS-28.

3.3- Estudo 3: ensaio clínico biomarcadores

O tratamento periodontal não cirúrgico (TPNC) reduz os níveis salivares e plasmáticos de RANKL, OPG, RANKL/OPG, survivina, concentração de ACPAs e Anti-CarP promovendo uma melhora na atividade clinica da AR e na condição periodontal.

A saliva e o sangue possuem níveis de quantificação dos biomarcadores RANK, OPG, RANKL/OPG, Survivina homólogos entre si.

3.4- Estudo 4: ensaio clínico anti- CarP e ACPAs

O tratamento periodontal não cirúrgico (TPNC) reduz os níveis salivares e plasmáticos das concentrações de ACPAs e Anti-CarP promovendo uma melhora na atividade clinica da AR e na condição periodontal.

4- METODOLOGIA

A metodologia, resultados e discussão desta pesquisa serão apresentados por meio de quatro artigos científicos intitulados:

ARTIGO 1. ESTUDO CASO-CONTROLE

ARTRITE REUMATÓIDE ASSOCIADA A PRESENÇA, GRAVIDADE E EXTENSÃO DA PERIODONTITE: UM ESTUDO CASO- CONTROLE

ARTIGO 2. ENSAIO CLÍNICO MICROBIOLÓGICO

ENSAIO CLÍNICO RANDOMIZADO CONTROLADO SOBRE O EFEITO DO TRATAMENTO PERIODONTAL NÃO-CIRÚRGICO EM INDIVÍDUOS COM ARTRITE REUMATÓIDE: ACHADOS CLÍNICOS E MICROBIOLÓGICOS

ARTIGO 3: ENSAIO CLÍNICO BIOMARCADORES

A TERAPIA PERIODONTAL NÃO CIRÚRGICA DIMINUI A GRAVIDADE DA ARTRITE REUMATOIDE E OS NÍVEIS PLASMÁTICO E SALIVAR DE RANKL E SURVIVINA: UM ESTUDO CLÍNICO PILOTO

ARTIGO 4. ENSAIO CLÍNICO ANTI-CARP E ACPAs

ANTICORPOS CONTRA PROTEÍNAS CARBAMILADOS E ANTICORPOS CONTRA PEPTÍDEOS CITRULADOS CÍCLICOS EM INDIVÍDUOS COM ARTRITE REUMATÓIDE E PERIODONTITE: UM ENSAIO CLÍNICO CONTROLADO

ARTIGO 1

RHEUMATOID ARTHRITIS ASSOCIATED WITH THE OCCURRENCE, SEVERITY AND EXTENSION OF PERIODONTITIS: A CASE-CONTROL STUDY

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Running title: Rheumatoid arthritis and periodontitis.

ARTIGO PUBLICADO NO JOURNAL OF CLINICAL AND EXPERIMENTAL DENTISTRY- QUALIS A-3

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Abstract

Background: Emerging evidence pointed to a potential association between periodontitis (PE) and rheumatoid arthritis **(**RA), based on shared characteristics and similarities in risk factors, immunogenetics and pathways of tissue destruction. The aim of this study was to evaluate the potential association between RA and PE, as well as the influence of risk variables in this association.
Methods: The present case-control study comprised 471 individuals (157 cases with RA and 314 controls) that underwent a full-mouth periodontal examination. The association between risk variables and the occurrence of AR and PE were evaluated through univariate and multivariate logistic analysis.
Results: Higher frequency ($p<0.001$), severity ($p=0.006$) and extension ($p=0.018$) of PE was observed among the cases when compared to controls. Variables retained in the final multivariate models for the occurrence of PE were: lower number of teeth, smoking, no use of dental floss, ≥4 daily toothbrushing and RA; for the occurrence of RA were: higher age, female gender, smoking, alcohol use and PE. It is important to stress that RA (OR=2.53; 95%CI 1.24–3.86; $p<0.001$) was retained in the model for PE, and PE (OR=3.12; 95%CI 1.47–4.26; $p<0.001$) was retained in the model for RA.
Conclusions: The present study demonstrated a high frequency of PE among individuals with RA and an important association among the occurrence, severity and extension of PE and RA and smoking.

Keywords: case-control study, risk factors, periodontitis, rheumatoid arthritis.

INTRODUCTION

Periodontitis (PE) is characterized by a microbiologically associated inflammation (1). Its pathogenesis is the outcome of complex interactions between periodontal pathogens and host immune response, having molecular pathways leading to the activation of host-derived proteinases and resulting in the migration of the junctional epithelium, subsequently destroying the periodontal attachment (2).

Several studies have demonstrated evidence of the association between PE and several systemic conditions, including diabetes, obesity, cardiovascular diseases, pregnancy disorders and rheumatoid arthritis (RA) (3,4). The underlying biological plausibility is based on the concept that PE inflammation and the periodontal microbiome contribute to the global burden of systemic inflammation at a level that affects the occurrence, severity and progression of other chronic inflammatory conditions (5).

RA is a chronic autoimmune disease that causes a breakdown of self-tolerance, chronic inflammation and debilitating joint destruction (6), compromising synovial fluids, joint cartilage and bone integrity (7). The etiology of RA remains uncertain, but the activity of periodontal pathogens has been linked to the production of RA auto-antibodies (8). RA is characterized by the presence of rheumatoid factor and anti-citrullinated protein / peptide antibodies (ACPAs) (9).

Some studies (10-17) pointed to a potential association between PE and RA, based on shared characteristics and similarities in risk factors, immunogenetics and pathways of tissue destruction (18,19). These diseases essentially differ, being RA an autoimmune disease while PE is an infectious disease (2).

A specific hypothesis suggests that certain oral bacteria can induce protein citrullination under the action of the enzyme peptidyl arginine deiminase, existing in both *Porphyromonas gingivalis* and inflammatory cells. Thus, *P. gingivalis* can play a crucial role in breaking immunological tolerance by inducing the citrullination of host proteins, converting them into autoantigens (19). These modified proteins, under a common immunogenic background, can

be recognized by the immune system, triggering an inflammatory process that is associated with the clinical manifestations of both diseases. ACPAS are the most suitable biomarkers for tracking RA and are a common serological finding in RA and PE (19-21). ACPAs are associated with radiographically detectable damage and extra-articular manifestations, found years before the beginning of clinical RA (20).

A recent systematic review of observational studies revealed that, although most reported an association between RA and PE, others claim that this association may be related to biases in PE assessment and to the absence of treatment for confounding factors. In addition to conflicting data, the quantitative analysis showed a high heterogeneity among studies, leading to the need for further studies (9).

The present study focused on evaluating the periodontal condition and the clinical and epidemiological aspects of the association between PE and RA through a case-control study.

MATERIALS AND METHODS

Sampling strategy

The present case-control study comprised a convenience sample of 725 individuals, male and female, age ranging from 35 to 65 years old, with no antibiotic use for a 3-month period prior to the study entry and a minimum of 6 months since the last periodontal treatment. Individuals were selected from those who were under medical treatment at the Rheumatology Outpatient Clinic from the Hospital das Clínicas of the Federal University of Minas Gerais, Belo Horizonte – Brazil.

Sample size was determined through the Fleiss Method with continuity correction, considering a significance level of 0.05, 90% study power, and a 1:2 proportion between case and controls. Therefore, assuming an expected prevalence of PE of 40% among cases with AR and 25% among

healthy controls, a number of 122 cases and 244 controls were determined to be necessary.

During the period of data collection, from September 2018 to January 2020, 725 individuals were interviewed and available for periodontal examination. After applying the exclusion criteria, 254 individuals were excluded (diabetes n = 68; other concomitant rheumatic diseases n = 38; orthodontic appliances n = 4; less than 12 teeth or presence of full dentures = 113; HIV positive n = 3; impossibilities for undergoing periodontal examination n = 28), thus 471 individuals were examined and determined to be eligible for the study. Final sample comprised 157 individuals with RA (cases) and 314 without RA (controls). Control group was composed by appointment companions, relatives, and staff members from the study hospital unit, all of them without RA. Sampling strategy is shown in Figure 1.

The present study was approved by the Research Ethics Committee from the Federal University of Minas Gerais, Belo Horizonte – Brazil (CAAE#48355915300005149). All individuals provided written informed consent before enrolling in the study.

Data collection

Interviews were performed through a structured questionnaire, recording the following data: gender, age, general health status, alcohol consumption (frequency / amount), smoking and oral hygiene habits. It was also collected from the medical records of all case individuals data on RA activity through the Disease Activity Score (DAS-28) (22). The most common medications used are methotrexate, vitamin D, calcium, folic acid, leflunomide e prednisone.

In relation to smoking, individuals were determined to be smokers if they had smoked ≥100 cigarettes throughout life and were still smoking during the study examination period; those who had smoked <100 cigarettes throughout life and were not smoking at the study examination period were determined to be non-smokers (23).

Periodontal clinical examination

Periodontal clinical examination was performed with a manual periodontal probe.1 The following periodontal parameters were evaluated and recorded from all present teeth, except from third molars, at 4 sites (mesial, distal, buccal e lingual): (i) probing depth (PD); (ii) clinical attachment level (CAL); (iii) bleeding on probing (BOP) (iii).

Intra and inter-examiner agreement

Periodontal examinations were performed in 12 individuals by 2 trained and expert periodontists (M.F.M and F.O.C). Intra and inner-examiner agreement for PD and CAL parameters revealed kappa values higher than 0.88 and intraclass correlation coefficient higher than 0.90.

Periodontitis definition and staging

Individuals were classified according to periodontitis stages and defined as periodontitis cases from stage II: Stage II: ≥ 2 interproximal sites with CAL of 3 to 4 mm, PD ≤5 mm, horizontal bone loss up to the coronal third (15% to 33 %) and without tooth loss due to periodontitis. Total periodontitis were defined as the sum of stages II, III and IV, according to the criteria by Tonetti et al. (1)

Rheumatoid arthritis diagnosis

Diagnosis and severity of RA (early, moderate and severe stages) were determined by the rheumatologic medical team of the study hospital, according to the criteria stablished by the American College of Rheumatology (24). These include having at least 4 of the following characteristics for at least 6 weeks: morning joint stiffness lasting for at least 1 hour; arthritis in at least

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three joint areas; arthritis of hand joints and wrists, proximal interphalangeal joints (middle finger joint) and metacarpophalangeal joints (between fingers and hand); symmetrical arthritis (in the left and right wrist, for example); presence of rheumatoid nodules; presence of rheumatoid factor in the blood and radiographic changes: joint erosions or decalcifications located on hand and wrist radiographs. In addition, RA activity was assessed using the DAS-28 method (22) in stages of activity, i.e., remission, early, moderate and high activity.

Statistical analysis

Groups were initially compared in relation to the following variables: gender, age, smoking (non-smokers / former-smokers and smokers), alcohol consumption, oral hygiene habits, DAS-28 scores and use of medications by the Chi-square and Mann-Whitney tests and Spearman correlation, when appropriate. Regarding the periodontal parameters (PD, CAL, BOP), the values per individual were obtained by the sum of the measures of all periodontal sites and expressed as means and / or percentages.

The distribution of independent variables by PE stages, odds ratio (OR) and their 95% confidence intervals (CI) were calculated. The effect of variables of interest on the occurrence of PE and RA was assessed using the multivariate logistic regression. The Generalized Estimation Equations method was used to obtain marginal logistic models that directly incorporate the correlation between the measures of the same sample unit. Variables with a p-value <0.25 in the bivariate analysis were then included in each multivariate model (non-cases (0) and cases (1)) and removed manually step by step until the likelihood ratio test indicated that no variable should be removed. All variables included in the final multivariate models were determined to be independent by assessing their collinearity. The quality of the models was determined by measures of sensitivity, specificity, area under the ROC

(Receiver Operating Characteristic) curve, R^2 (Nagelkerke) and Hosmer-Lemeshow test. All analyzes were performed using statistical software R.2

RESULTS

Sample comprised 471 individuals, being 145 male and 326 female, with mean age of 47.39±5.18 years old in the control group and of 54.1±11.45 years old in the case group. The frequency of alcohol use among controls and cases was 54.2% and 20.7%, respectively (p<0.001). Moreover, individuals with RA showed significantly higher mean age (p<0.001), smokers (p<0.001) and less use of dental flossing (<0.025) and less alcohol consumption (p<0.001). Brushing frequency did not show significant differences between the groups (Table 1).

A high frequency of total PE (stages II + III + IV) was observed among cases (53.8%) when compared to controls (30.1%) (p<0.001). Individuals with RA presented a 2.64 higher chance of having PE (unadjusted OR = 2.64; 95%CI 1.78–3.94; p<0,001). In addition, the severity (p = 0.006) and extension (p = 0.018) of PE were higher among RA individuals (Table 2).

Regarding periodontal clinical parameters, individuals with RA presented significantly worse parameters than individuals without RA, that is, lower number of teeth, higher average of sites with BOP, higher PD and CAL, higher percentage of sites with PD 4–6 mm and PD ≥6mm, higher percentage of sites with CAL ≥3mm and CAL ≥5mm (Table 2).

Table 3 shows the final logistic regression models and respective variables significantly associated with: (i) the occurrence of PE (model 1) – RA, lower number of teeth, smoking, no use of dental flossing and protective effect for ≥4 daily toothbrushing; (ii) the occurrence of RA (model 2) – PE, older age, female sex, smoking and a protective effect of alcohol use. It is noteworthy that PE (OR = 3.12; 95%CI 1.47–4.26; p<0.001) remained in the model for AR and AR (OR =2.53; 95%CI: 1.24- 3.86; p<0.001) remained in the model for PE.

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[2] Software R originally created by Ross Ihaka & Robert Gentleman, University of Auckland, New Zealand (version 3.6.3).

RA activity by stages (remission, early, moderate and high activity) was assessed through the DAS-28 method and correlated with PE. However, there was no significant correlation between DAS-28 scores and periodontal clinical parameters of PD, CAL and BOP, occurrence of PE, as well as in relation to the severity of PE ($p>0.05$; data not shown).

The use of different medications such as methotrexate, vitamin D, calcium, folic acid, leflunomide and prednisone by individuals with RA did not shown significant differences in relation to the occurrence or the severity of PE ($p>0.05$; data not shown).

DISCUSSION

The present study showed a higher occurrence of PE in individuals with RA when compared to controls (OR=2.64; 95% CI: 1.78–3.94; $p<0.001$). In addition, greater severity and extension of PE were also observed. This association remained in the multivariate models for both PE and RA.

It is hypothesized that both conditions share common characteristics and pathogenic similarities in relation to immunogenetics and tissue destruction pathways (21). They are chronic inflammatory diseases that share mutual risk factors for susceptibility, such as HLA-DRBI alleles and smoking. In addition, the role of *P. gingivalis* and its enzyme PAD, that is capable of generating citrullinated epitopes that are recognized by ACPAs, has been proposed as a link between PE and RA. Recently, two isoforms of the peptidyl arginine deiminase family (PAD2 and PAD4) and citrullinated proteins have been identified in inflamed periodontal tissues, reinforcing the hypothesis that periodontal citrullination may play an important role in the etiopathogenesis of RA (19,21).

Observational studies have shown that patients with RA have an increased occurrence of PE compared to healthy individuals without RA (11,19,25-30). Additionally, the frequency of RA was higher in patients with PE compared to those without PE (14,15). However, findings from other population-based studies have failed to show any association (10,12,26,30) or showed a weak association (13) between these two conditions.

Potential frequent biases can be observed in several studies, such as the use of retrospective data obtained from medical records, small samples, indexes or radiographic exams to evaluate the occurrence of periodontitis, self-reported information about periodontal status and RA. Thus, additional studies that control these factors are necessary.

In the present study, individuals with RA had significantly worse periodontal status when compared to controls, presenting a higher average of sites with BOP, higher mean PD and CAL and lower number of teeth. In the study by Rosamma et al. (28) and Zhao et al. (29), worse clinical periodontal parameters were also observed among individuals with RA.

DAS-28 is the gold standard method to measure RA activity (22). In this present study, there was no significant correlation between DAS-28 scores and periodontal clinical parameters (PD, CAL and BOP) in relation to the occurrence and severity of PE. Different studies (28-30) have also found no association between rheumatoid disease activity and the occurrence and severity of PE. However, in the study by Choi et al. (31), BOP was correlated with DAS-28 scores, although no correlations for PD and CAL.

On the other hand, in the study De Smit et al. (27), individuals with RA and severe PE had higher DAS-28 scores when compared to individuals with RA and moderate PE or even without PE. The presence of PE was also associated with greater disease activity according to DAS-28 scores by Mikuls et al. (11) and Zhao et al. (29).

In a recent systematic review and meta-analysis, there was no substantial effect of RA on PD and CAL among individuals with PE when compared to controls. However, it was demonstrated that there is consistent evidence suggesting that PE is associated with worse clinical activity of RA, as assessed by DAS-28 scores, whereas individuals with RA do not worsen clinical parameters of PE. It is important to note that a high heterogeneity was observed among the 6 meta-analyzed studies (26).

Depending on the activity of RA and on the individual response, the use of several medications is necessary to minimize tissue damage and control the disease. In past decades, it has been suggested that the use of anti-inflammatory drugs and corticoids could decrease the severity and occurrence of PE (30).

However, this issue was not sustained over the years. As few studies have approached this topic, the present study investigated the most used medications among individuals with RA such as methotrexate, vitamin D, calcium, folic acid and prednisone. Findings showed that the use of any of the mentioned drugs did not infer significant differences in relation to the occurrence and severity of PE. Ziebolz et al. (30) reported that drugs used to treat RA may be associated with periodontal inflammation, but without differences in the severity of PE.

The multivariate analysis for the occurrence of PE retained the following variables in the final logistic model: RA, lower number of teeth, smoking, no dental flossing and a protective effect for ≥4 daily toothbrushing. Corroborating previous findings, variables such as smoking and worse oral hygiene habits can contribute to the activation of systemic triggers for a prolonged period, leading to immunological changes (exacerbation in the expression of cytokines), as well as endocrine and behavioral disorders, that can predispose to greater susceptibility to both diseases (3,19).

Tooth loss is considered the final outcome of oral diseases that affect periodontal and dental tissues, affecting the oral health-related quality of life. In this present study, individuals with RA presented significantly lower number of teeth than controls. Similar results have been previously reported (19,28,29).

The multivariate analysis for the occurrence of RA retained the following variables in the final logistic model: PE, smoking, female gender, older age, and a protective effect of lower alcohol use. Smoking, female sex and age are known to be strong risk factors for the occurrence of RA. According to Hussain et al. (26), the reduction in the frequency of alcohol use revealed significant improvements in pain and quality of life in individuals with RA.

It is important to highlight that in both multivariate models, PE (OR = 3.12; 95% CI: 1.47–4.26; $p<0.001$) remained in the model for AR and AR (OR = 2.53; 95% CI: 1.24–3.86; $p<0.001$) remained in the model for PE. This reinforces the association between these 2 conditions. In addition, the presence of smoking in both models also reinforces the hypothesis that common risk and susceptibility factors are shared between them.

Some limitations must be attributed to the present study. The convenience sample may have some impact on the external validity of the

results. Due to temporality, the case-control study does not detect any temporal influence between RA and PE, as well as direction of the association cannot be clearly established. Another issue is that this study design is susceptible to selection bias, since exposure, disease occurrence and its outcomes is recorded at the moment the patient is recruited for the study. However, in order to minimize these factors, all individuals in sample of the present study were recruited from a single center with homogeneous characteristics.

On the other hand, advantages can also be cited for the present study as: (i) the high number of individuals in the sample, increasing the statistical power of the study; (ii) the diagnosis of RA by specialized doctors; (iii) the full-mouth periodontal examination with a robust periodontitis definition, since it is recognized that the quality of the periodontal data and the criteria for PE definition can strongly impact the results of the association studies (3).

Future studies with different samples and prospective designs, including microbiological and immunological analyzes, would be of paramount importance to provide more accurate conclusions about the periodontal status of individuals with RA and the association between PE and RA.

The present study reported a higher occurrence, severity and extension of PE in individuals with RA. A strong association between RA and PE was observed after adjusting for important confounding variables in multivariate models, including smoking as a common risk factor shared between both conditions.

Availability of Data and Materials

The authors consent to the use of this article and its data without restriction, provided that the original authors are cited. Data are available from the corresponding author at a reasonable request.

Conflict of Interes: The authors declare no conflicts of interest.

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Table 1. Characterization of the sample.

Variables	Total sample (n=471)	Control group (n=314)	Rheumatoid Arthritis group (n=157)	p	OR (95% CI)
Age (mean ± s.d.)	49.13 ± 7.88	47.39± 5.18	54.1 ± 11.45	**<0.001***	-
Gender (n (%))					
Male	145 (30.8%)	119 (38%)	32 (20.3%)		1.00
Female	326 (69.2%)	195 (62%)	125 (79.7%)	**<0.001****	2.38 (1.52-3.77)
Smoking (n (%))					
No	400 (84.82%)	278 (88.6%)	116 (74.1%)		1.00
Yes	71 (15.18%)	36(11.4%)	41 (25.9%)	**<0.001****	2.72 (1.65-4.49)
Alcohol consumption (n (%))					
No	256 (54.46%)	144 (45.8%)	125 (79.3%)	**<0.001****	1.00
Yes	215 (45.54%)	170 (54.2%)	32 (20.7%)		0.21 (0.13- 0.33)
Dental flossing (n (%))					
No	240 (50.9)	176 (56.0)	73 (46.3)	**0.025****	1.00
Yes	231 (49.1)	138 (44.0)	84 (53.7)		1.47 (1.0-2.15)
Toothbrushing (n (%))					
≤1 daily	0 (0%)	0 (0.0%0	0 (0.0%)		-
2-3 x daily	350 (74.5%)	238 (75.7%)	115 (73.2%)		1.0
≥4 daily	121 (25.5%)	76 (24.3%)	42 (26.8%)	0.273**	0.87 (0.56-1.36)

s.d.= standard deviation; *Mann-Whitney test; **Chi-square test; Significant values are shown in bold.

Table 2. Periodontal clinical parameters of the sample.

Variables	Rheumatoid Arthritis group (n = 157)	Control group (n = 314)	Crude OR (95%CI)	p
Periodontitis total (Stage II + III + IV)				
No	73 (46.2%)	219 (69.9%)	2.64 (1.78-3.94)	**<0.0001***
Yes	84 (53.8%)	95 (30.1%)		
Stage of periodontitis	n=84	n=95		
Moderate (stage II)	51 (60.6%)	65 (68.0%)	1.29 (0.91-1.85)	0.071*
Severe and advanced (stage III or IV)	33 (39.4%)	30 (32.0%)	1.82 (1.15-2.92)	**0.006***
Extension of periodontitis				
Localized	59 (70.3%)	71 (74.4%)	1.40 (0.99-1.97)	0.025*
Generalized	25 (29.7%)	24 (25.6%)	1.70 (1.03-2.89)	**0.018***
Number of teeth (%)	23.37 ± 2.86	24.63 ± 3.02	22.12 ± 2.61	0.035**
BOP (%)	54.1 ± 32.9	44.2 ± 31.6	-	**0.002****
PD (mm)	3.1 ± 1.9	2.3 ±1.3	-	**<0.001****
CAL (mm)	4.8 ± 1.8	4.0 ± 1.5	-	**<0.001****
% sites with PD <4mm	80.8 ± 18.9	83.0 ± 14.9	-	**<0.001****
% sites with 4-6mm	14.9 ± 7.5	13.8 ± 5.6	-	**<0.001****
% sites PD >6mm	4.3 ± 1.6	3.2 ± 1.2	-	**<0.001****
% sites with CAL <4mm	32.6 ± 8.7	30.7 ± 6.1	-	**<0.001****
% sites with CAL ≥5mm	21.3 ± 22.1	18.2 ± 19.1	-	0.031**

BOP = bleeding on probing; PD = probing depth; CAL = clinical attachment level; *Chi-square test; **Mann-Whitney Test; Significant values in bold.

Table 3. Final multivariate logistic models for the occurrence of periodontitis and rheumatoid arthritis.

Model 1: Final multivariate model for the occurrence of periodontitis total		
Variables	**OR (95% CI)**	**p**
Number of teeth	1.12 (1.26-1.81)	0.019
Smoking	2.60 (1.13-5.21)	0.032
Toothbrushing ≥4 daily	0.14 (0.03-0.39)	<0.001
No use of dental flossing	1.16 (1.0 – 1.61)	0.039
Rheumatoid Arthritis	2.53 (1.24- 3.86)	<0.001
Model 2: Final multivariate model for the occurrence of rheumatoid arthritis.		
Variables	**OR (95% CI)**	**p**
Smoking	4.15 (1.19 -14.79)	0.002
Female sex	2.19 (1.13 - 3.21)	0.025
Alcohol use	0.19 (0.05 - 0.49)	0.001
Age	1.12 (1.07-1.19)	<0.001
Periodontitis total	3.12 (1.47-4.26)	<0.001

Model 1: (Hosmer-LemeshowTest: 0.923; Pseudo R^2: 37.12%; AUC: 0.772; Sensibility: 0.74; Especifity: 0.721). **Model 2:** (Hosmer-LemeshowTest: 0.751; Pseudo R^2: 67.94%; AUC: 0.917; Sensibility: 0.903; Especifity: 0.791).

ARTIGO 2

CLINICAL AND MICROBIOLOGICAL EFFECTS OF NON-SURGICAL PERIODONTAL TREATMENT IN INDIVIDUALS WITH REUMATHOID ARTHRITIS: A CONTROLLED CLINICAL TRIAL

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Authors contribution

All authors have made substantial contributions to the conception and design of the study. MFM, MML, JRC and FOC have been involved in data collection and data analysis. MFM, LOMC, TAS, SCC, GAF, MML, JRC and FOC have been involved in data interpretation, the drafting of the manuscript and revising it critically and all have given final approval of the version to be published.

ABSTRACT

Objectives: The effect of periodontal treatment on clinical, microbiological and serological parameters of patients with rheumatoid arthritis (RA) are scarce and controversial. The aim of this study was to investigate the influence of non-surgical periodontal treatment on clinical periodontal status, subgingival bacterial levels of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia, Treponema denticola* and RA activity through a controlled clinical trial on individuals with RA and periodontitis (PE).
Methods: From a convenience sample, 107 individuals were considered eligible and consecutively allocated in 4 groups: 1) individuals without PE and RA (-PE-RA, n=30); 2) individuals without PE and with RA (-PE+RA, n=23); 3) individuals with PE and RA (+PE+RA, n=24); and 4) individuals with PE and without RA (+PE-RA, n=30). Full-mouth periodontal clinical examinations, microbiological analysis and Disease Activity Score (DAS-28) evaluations were performed at baseline (T1) and 45 days after non-surgical periodontal treatment (T2).
Results: At T1, individuals +PE+RA showed greater severity of PE than +PE-RA individuals. At T2, significant reductions were observed in all periodontal clinical parameters in both groups ($p<0.001$) with a significant reduction in DAS-28 in +PE+RA ($p=0.011$). Individuals +PE-RA and +PE-RA showed significant reductions for all bacteria ($p<0.001$). Additionally, *P. gingivalis* demonstrated an expressively significant reduction in +PE+RA ($p<0.001$).
Conclusions: Non-surgical periodontal treatment was effective on improving the clinical periodontal condition, improving the RA clinical status and reducing the presence of periodontal pathogens.
Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/).

Key words: bacteria; clinical trials; nonsurgical periodontal debridements; periodontitis; rheumatoid arthritis.

INTRODUCTION

Periodontitis (PE) is a chronic infectious inflammatory disease caused by anaerobic bacteria, leading to the destruction of teeth supporting tissues [1]. The disease results from the disturbance of homeostasis between the subgingival microbiota and the host's defenses in susceptible individuals [2]. This is due to different aspects of both innate inflammatory and immune responses.

In recent years, the association between immune-mediated inflammatory diseases and PE has been increasingly reported. Several studies point to the evidence of association between PE and several systemic conditions, including diabetes, obesity, cardiovascular diseases, pregnancy disorders and rheumatoid arthritis (RA) [1,2-4].

Biological plausibility is based on the concept that inflammation and periodontal microbiome contribute to the global burden of systemic inflammation at a level that affects its occurrence, severity and progression of other chronic inflammatory conditions [5]. This association does not imply causality and can be unidirectional or bidirectional, that is, periodontitis and other oral conditions could influence systemic diseases as well as some systemic diseases could also impact on periodontal outcomes [5].

It has long been recognized that RA and periodontitis share many common pathological features, such as chronic inflammation induced by pro-inflammatory cytokines and destruction of connective and bone tissues [6]. Moreover, previous findings give evidence to a relationship between the presence of inflammation associated with periodontal pathogens and the development of RA [5]. Thus, pathogens such as *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T. denticola, Prevotella intermedia, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus, Eiknella nodatum, Eiknella corrodens* and *Capnocytophaga species (Cs),* are sources of repeated transient systemic bacteremias, therefore increasing the susceptibility to RA through the metastatic spread of inflammation [6].

P. gingivalis, a common pathogen in PE, could citrullinate proteins / peptides in particular, inducing the autoimmune response and susceptibility of

individuals to RA [5,7]. This could be due to antibodies against citrullinated proteins / peptides being a common serological finding in both RA and PE [8-11].

Thus, strong epidemiological associations, as well as serological and clinical associations, have been observed between RA and PE [12-16]. Previous studies have demonstrated that the prevalence of RA is higher in patients with PE than in patients without PE and vice versa [3,17]. A systematic review by Calderaro *et al*. [18] included 4 articles analyzing the effect of non-surgical periodontal treatment on RA and showed that non-surgical periodontal treatment was associated with a significant reduction in RA activity, but evidencing high heterogeneity between the studies.

There are still conflicting results [13,19-25] when it comes to intervention studies on the effect of periodontal treatment on the clinical, microbiological, serological parameters of patients with RA. Therefore, although it is emerging, this effect is still controversial and further investigations are necessary, mainly through controlled clinical trials.

In this sense, the aim of the present study was to investigate the influence of non-surgical periodontal treatment on the clinical periodontal condition, bacterial levels of *A. actinomycetemcomitans*, *P. gingivalis*, *T. forsythia*, *T. denticola* and RA activity through a controlled clinical trial on individuals with RA and PE.

METHODS

Study design and ethical considerations

The present controlled clinical trial was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE #03128012.0.0000.5149) and registered in the Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/). This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Participants were informed about the research objectives and signed an informed consent form prior to entering the study.

Study sample and sampling strategy

Individuals with a diagnosis of PE from the waiting list of the School of Dentistry of the Federal University of Minas Gerais and with a diagnosis of RA from the Rheumatology Service of the Hospital das Clínicas of the Federal University of Minas Gerais were invited to participate in the present study. Individuals without any rheumatological and periodontal diseases, randomly selected among relatives, companions or employees of the respective reference centers were used as controls.

A sample size calculation was performed considering as the primary outcome the reduction in probing depth (PD) and gain in clinical attachment level (CAL) from a previous study [26]. The reduction in bacterial load was considered as the secondary outcome and was also based on previous mean bacterial counts [27]. Considering a significance level of 5%, 80% study power and a minimum difference of 15% between study groups in relation to the reduction in PD (mean values), it was determined to be necessary an estimated number of ~20 individuals per group. It is noteworthy that the variation coefficient for bacterial counts in the present study was ~15%, indicating the precision of the study result [27].

From a convenience sample of 327 individuals that were interviewed and examined from September 2018 to January 2020, after adopting the exclusion criteria (figure 1), 107 non-smokers and non-diabetics individuals were considered eligible and consecutively included until the compounding of the following 4 groups: group 1 (control) – individuals without PE and RA (-PE-RA, n=30); group 2 – individuals without PE and with RA (-PE+RA, n=23); group 3 – individuals with PE and RA (+PE+RA, n=24); and group 4 – individuals with PE and without RA (+PE-RA, n=30). Plus, an additional margin of 20% in the sample number per group was added due to the risk of future losses from follow-up exams.

Interviews were conducted by applying a standardized questionnaire aiming to collect the following data: gender, age, general health conditions and oral hygiene habits. Data on RA activity was also collected from participants' medical records by the Disease Activity Score (DAS-28) method [28].

After collecting these data, all individuals underwent a full-mouth periodontal examination, keeping records of periodontal clinical parameters, which were evaluated in all teeth at four sites (mesial, distal, buccal and lingual): (1) probing depth (PD); (2) clinical attachment level (CAL); (3) bleeding on probing (BOP). A manual periodontal probe model[3] clinical mirror and gauze were used.

Periodontitis definition and staging

Individuals were defined as periodontitis cases and classified by staging from Stage II – those with at least ≤2 interproximal sites with CAL of 3 to 4mm, with PD ≥5mm, with a pattern of horizontal bone loss up to the coronal third (15% to 33%) and without tooth loss due to periodontitis according to the criteria defined by Tonetti et al. [29].

Reumathoid arthritis diagnosis

The diagnosis and severity of RA (mild, moderate or severe forms) were established by a group of rheumatologists associated with the Rheumatology Service of the Hospital das Clínicas of the Federal University of Minas Gerais according to criteria of the American Society of Rheumatology [30]. Additionally, RA activity was assessed by using the DAS-28 method [28] in stages of activity, i.e., remission, mild, moderate and severe activity.

In clinical practice, judgement on disease activity is formed from a combination of information. DAS-28 is a quantifiable disease activity index formed from laboratory and clinical variables, and the overall impression of the patient. Hence, the DAS-28 evaluation included 28 joint counts, plus the erythrocyte sedimentation rate (ERC) or C-reactive protein (CRP) and a general health assessment (VAS). In clinical practice, this index gives an opportunity to compare the efficacy of treatments in the management of RA. The EULAR (European League Against Rheumatism) response criteria are measures that

[3] UNC-15 (PCPUNC-15, Hu-Friedy, Chicago, IL, USA.

classify individuals depending on the extent of change and the level of disease activity reached in response of treatment (Figure 2.) Index calculation was performed in an application available at *http:/www.4s-dawn.com/DAS28/*.

Periodontal treatment

Individuals in the -PE-RA and -PE+RA groups (individuals without periodontitis) were handed toothbrushes and instructions for proper oral hygiene after periodontal examinations.

In the +PE+RA and +PE-RA group, scaling and root planing procedures were performed using a full-mouth disinfection (FMD) technique in a single stage (24 hours) divided into two sessions (60 min. per session) on two consecutive days , with the inclusion of chlorhexidine gel (CX) irrigation (1%) in the periodontal pockets after mechanical debridment, brushing of the tongue for 1 min. with CX gel (1%), and mouthwash at the beginning and end of each session with 0.12% CX for 30 seconds (gargle form in the last 10 seconds)[31]. Antibiotic therapy was not used as an adjunct to FMD procedures.

Periodontal clinical examinations and microbiology analysis were performed at T1 (baseline) for the 4 groups. After 45 days (T2), new periodontal clinical examinations were performed for all groups, however the microbiological analysis (samples collected always from the same teeth and sites) was only performed again in +PE+RA and +PE-RA groups. DAS-28 was collected again after 45 days for the +PE+RA group. A flowchart with the design study is shown in Figure 1.

Examinations and treatment by FMD were performed by 3 properly trained and calibrated examiners (MFM, FOC and JRC). When an exam was performed by one of these examiners, treatment and the second assessment was performed by the two others. Weighted kappa agreement tests for clinical parameters of interest (PD and CAL) revealed intra and inter-examiner values greater than 92%.

FMD procedures were performed by using Gracey and McCall curettes[4]. An independent set of scrapers was used for each group, being sharpened with each use and discarded after six consecutive uses.

Microbiological analysis

As described in our previous study, subgingival plaque samples were collected [32]. Supragingival dental plaque was removed using a sterile curette, and a sterile paper point (fine, Johnson and Johnson, New Brunswick, NJ, USA) was inserted into the gingival sulcus/periodontal pocket and kept there for 10 seconds. Then, paper point was placed in a vial containing 1.0 ml of phosphate-buffered saline (pH7.4), inserted in a minitube and kept on ice. To obtain bacterial dispersion, a vortex mixer at maximum speed was used for 1 min and stored in a freezer at -80°C until further analyses.

Following the manufacturer's specifications, the genomic DNA (gDNA) was extracted and purified using a commercial Genomic DNA Mini Kit (Life Technologies, Carlsbad, CA, USA).The total microbial count of *Tannerella forsythia, Porphyromonas gingivalis, Treponema denticola* , and *Aggregatibacter actinomycetemcomitans* was performed by quantitative real-time polymerase chain reaction (qPCR) using a set of TaqMan (Life Technology, Carlsbad, CA, USA) primers/probes in Real-Time PCR System, following manufacturer's instructions. The qPCR conditions were: 50º C for 2 min, 95º C for 10 min, 40 cycles of 95º C for 15 s, and 60º C for 1 min. Figure 3 lists the primers and probes used in the study.

Statistical analysis

Sample characterization data, periodontal clinical parameters, microbiological exams and DAS-28 scores were analyzed in the baseline (T1) for all groups and 45 days after treatment (T2) for +PE+RA and +PE-RA groups using the Chi-square, Kruskal- Wallis, Mann-Whitney and Wilcoxon tests, when

[4] Hu-Friedy, Chicago, IL, USA.

appropriate. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. When there was a significant difference between the means of at least two groups, pairwise comparisons were performed using the Nemenyi post-hoc test. Microbial counts were log-transformed, and a Q-Q plot of residual values showed acceptable levels of normality. Spearman's correlation test was performed to assess the correlation between bacterial levels and periodontal clinical parameters. Since individuals with RA and controls were not directly matched, it was not necessary to take into account the uneven size of the groups in the statistical comparison at the group level. All analyzes were performed using the statistical software R.[5]

RESULTS

Table 1 shows the demographic variables, DAS-28 under age and gender, reflecting some peculiarities of both diseases, such as older age in the groups with PE and high frequency of women in the groups with RA ($p<0.001$). Regarding periodontal parameters, comparisons of interest (+PE+RA versus +PE-RA) demonstrated that the +PE+RA group had higher mean values of PD ($p<0.001$), CAL ($p<0.001$) and % of patients with PD 4-6mm ($p<0.001$) than the +PE-RA group, with no differences in the number of present teeth ($p=0.077$). These findings reveal a greater severity of PE in +PE+RA individuals and in relation to +PE-RA, at T1.

At T1, microbiological findings of greatest interest comparing the 4 groups were: the absence of significant differences in bacterial counts between +PE+RA and +PE-RA groups. There were no significant differences in bacterial counts between -PE+RA and -PE-RA individuals. However, these groups showed significantly lower bacterial counts than+PE+RA and +PE-RA groups (Table 1).

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[5] Software R originally created by Ross Ihaka & Robert Gentleman, University of Auckland, New Zealand (version 3.6.3).

At T2, there was no significant change in all periodontal clinical parameters for -PE+RA and -PE-RA groups. Periodontal clinical conditions after non-surgical periodontal treatments (from T1 to T2) for the +PE+RA and +PE-RA groups is shown in Table 2. In the intragroup analysis, significant reductions in the means of PD, BOP and CAL in +PE+RA and +PE-RA were observed, showing efficiency in non-surgical periodontal treatment in both groups. As for reductions in the severity of PE, they were significantly observed in +PE+RA (% of sites with PD 4-6mm from 31.3% to 13%; PD >6mm from 3.3% to 0.6%; $p<0.001$) and in the +PE-RA (% of sites with PD 4-6mm from 18.6 % to 2.1%; PS >6mm from 4.0 % to 0.6%; $p<0.001$). However, in intergroup comparisons at T2, the +PE-RA group showed significantly better periodontal clinical parameters than those observed in the +PE+RA group (Table 2).

Comparative microbiological findings for the +PE+RA and +PE-RA groups after non-surgical periodontal treatment are shown in Table 3. In the intragroup analysis, +PE+RA and +PE-RA groups showed significant reductions in the mean values between T1 and T2 for all bacteria ($p<0.05$). The intergroup analysis at T2 showed no significant differences in the counts of *T. denticola* and *T. forsythia* between +PE+RA and +PE-RA ($p>0.05$), whereas *A. actinomycetemcomitans* showed a greater reduction in +PE-RA ($p<0.020$) and *P. gingivalis* an expressive and significant reduction in +PE+RA ($p<0.01$) (Table3).

In relation to DAS-28 scores at T1, PE-RA+ and +PE+RA groups presented values of 3.69 ± 1.25 and 4.34 ± 0.89, respectively, with significant differences between them ($p=0.024$). At T2, there was no change in DAS-28 for the -PE-RA+ group (3.65 ± 1.29) and a significant reduction was observed in +PE+RA individuals, with the scores reduced to 3.12 ± 0.71 ($p=0.011$). The change in DAS scores from T1 to T2 (1.22) in the +PE+RA group was determined to be moderate according to the EULAR criteria (Figure 2), revealing a beneficial effect of non-surgical periodontal treatment on serological levels (reduction in CRP levels) and symptoms of AR. Moreover, DAS-28 showed significant positive correlations with PD ≥4mm (0.13; $p<0.001$) and BOP (0.06; $p=0.005$), but with no significant correlations with bacterial levels.

Table 4 shows the correlation among bacterial levels and periodontal clinical parameters. It was observed that in both groups (+PE+RA and +PE-RA)

all bacteria showed significantly positive correlations for BOP, PD and CAL ≥4mm, worse periodontal clinical parameters generally correlated with higher bacterial counts and vice versa. It highlights the strong correlations between *P. gingivalis* and all periodontal clinical parameters in +PE+RA group ($p<0.001$).

The use of different medications such as methotrexate, vitamin D, calcium, folic acid, leflunomide and prednisone by individuals with RA showed no significant differences regarding the occurrence or severity of PE ($p>0.05$; data not shown).

DISCUSSION

Findings from the present study have shown a beneficial effect of non-surgical periodontal treatment in reducing RA symptomatology, demonstrated by the significant reduction in DAS-28 scores. This benefit was also extended to serological levels (lower levels of CRP). Furthermore, non-surgical treatment reduced levels of periodontal pathogens and significantly promoted improvements in all clinical periodontal parameters among individuals with PE with and without RA, with a superior improvement in individuals with PE and RA.

The idea underlying this study was not necessarily causality but rather association in a syndemic basis. Syndemic theories focus on concurrent or sequential health problems and the analysis of mutually aggravating interaction between them, including their consequences and management. Therefore, the main focus was the effects of periodontal treatment on clinical changes in DAS-28 scores, as well as improvements in periodontal clinical parameters. DAS-28 scores can be considered an important patient-centered outcome when managing AR.

This study revealed a greater severity and extension of PE in individuals with PE and RA at T1 in relation to those with PE and without RA as well. These findings are corroborated by previous studies [16,23,24,33], in which worse clinical parameters have also been reported in individuals with RA. It was also observed significant improvement in periodontal parameters after periodontal treatment in both +PE+RA and +PE-RA groups, revealing the efficiency of non-surgical periodontal treatment. However, the +PE-RA group

showed significantly better periodontal status at T2 when compared to those observed in the +PE+RA group.

Periodontal treatment was performed through the FMD technique due to logistical reasons and in order to avoid absenteeism. Since the presentation of this technique by Quirynen et al. in 1995, several studies have shown clinical and microbiological advantages of this technique over conventional scaling and root planning [31]. However, in a recent review, Pockpa et al. [34] reported an absence of significant differences between these two types of treatment, both being effective and able to be individually chosen by either dentists or patients.

Improvements in periodontal clinical outcomes followed by significant reduction in DAS-28 scores indicate that periodontal therapy could improve the clinical and serological status of RA. These findings have also been reported in previous studies [16,18.23.24]. Additionally, DAS-28 scores showed positive correlations with inflammatory periodontal parameters such as PD and BOP in the present study.

In a systematic review and meta-analysis on the effect of non-surgical periodontal treatment on RA by Calderaro et al. [18], it was demonstrated in 4 included articles that non-surgical periodontal treatment was associated with a significant reduction in DAS-28 scores, being beneficial in RA management. However, because of the few included studies, new controlled trials were determined to be necessary to confirm this finding.

There are several postulated mechanisms by which infections can trigger autoimmune diseases, but most evidence in animal models supports the idea that reactive immune responses cause autoimmunity due to molecular imitation between microbiological agents and autoantigens [13]. Initially, Rosenstein et al. [35] raised the hypothesis that *P. gingivalis*, an important periodontal pathogen, plays an important role in the pathogenesis of RA. *P. gingivalis* is a prokaryote that exclusively contains a peptidyl arginine deaminase enzyme required for citrullination and can induce an immune response to citrated autoproteins, being confirmed by several studies [9,10,35]. Citrulination alters the structure and function of proteins and has been demonstrated in several physiological and pathological processes [10].

Antibodies against citrullinated proteins (AACPs) are 95% specific and 68% sensitive to RA [8]. These antibodies can appear much before the clinical onset of RA [36] and are associated with a more destructive course of the disease than RA without detectable AACPs [11,37,38]. In addition, epidemiological associations indicate that periodontitis may contribute to the total inflammatory load, causing bacteremia and systemic inflammatory responses [39,40]. Confirming our findings, Zhao et al. [16] reported that reduced systemic inflammation could contribute to a better clinical course of RA.

Consistently with the improvement of periodontal parameters after non-surgical periodontal treatment, both groups (+PE+RA and +PE-RA) showed significant reductions for all bacteria. An interesting finding was a greater reduction in *A. actinomycetemcomitans* in the +PE-RA group and a greater reduction in *P. gingivalis* in the +PE+RA group. Additionally, there was a significant correlation between *P. gingivalis* and all periodontal clinical parameters in +PE+RA group. These results corroborate the hypothesis of *P. gingivalis* having an important impact on rheumatological status, as well as a contribution to a dysbotic biofilm with complex microbial interactions [38].

In general, worse periodontal clinical parameters significantly correlated with higher mean bacterial counts and vice versa. These findings revealed the efficiency of non-surgical periodontal therapy in reducing specific periodontal pathogens, being of great importance, as it is known that this reduction is accompanied by major improvements in the severity of periodontal clinical parameters and minimizes the need for surgical procedures [34].

Studies have reported higher antibody titers against *P. gingivalis* in RA patients and a positive correlation with AACPs [36,37], suggesting that infection by this periodontal pathogen may play a role in the risk of RA progression. de Scher et al. [41] evaluated the complete subgingival microbiota by pyrosequencing and detected a higher frequency of *P. gingivalis* in individuals with newly diagnosed and never treated RA compared to patients with established RA or healthy controls. This present study showed *P. gingivalis* as having a significant reduction between T1 and T2 for the treated groups. Based on the above findings, we can hypothesize an additional beneficial effect for individuals with PE and RA.

Recently, Perriconi et al. [11] has reported the pathogenic role of *P. gingivalis* in rat models in which RA was triggered or had worsened in infected animals. *P. gingivalis* showed its harmful role not only by the induction of citrullination, but also by other important mechanisms, including the induction of NETosis, osteoclastogenesis and Th17 pro-inflammatory response, leading to bone damage and systemic inflammation.

On the other hand, periodontal infection by *A. actinomycetemcomitans* could be another mechanistic link to trigger autoimmunity in RA. Leukotoxins from these bacteria are capable of deregulating the activation of endogenous peptidyl arginine deiminase enzymes, catalysts of citrunilization in neutrophils, leading to the release of hypercitrunylated proteins, thus imitating the repertoire of citrullinated antigens found in the joint in individuals with RA [42,43].

However, this is a short-term study. Longitudinal studies, and even periodontal maintenance therapy should be conducted to assess a long-term effect of periodontal therapy for managing RA patients.

There is a consensus on the need to reduce the total inflammatory load in individuals with RA. Currently, RA management is based on early diagnosis, rigorous treatment and regular monitoring, with remission of the disease being the ultimate goal of treatment. Thus, the control of periodontal infection in patients with RA and PE can be an important therapeutic tool.

The present study can be considered an important starting point for further investigations on the effect of periodontal treatment in individuals with RA and its correlation with clinical, serological and microbiological parameters. In this sense, new studies with different populations and designs should be conducted, in order to confirm these findings that solidify the robustness of the scientific evidence of the association between periodontitis and RA. In addition, further studies focusing on molecular outcomes, especially citrullinated proteins, would better evaluate the biological link between periodontitis and rheumatoid arthritis.

CONCLUSIONS

Periodontitis can be a potential contributing factor for the worsening RA, while RA is associated with a greater severity of PE. Non-surgical periodontal treatment was effective in improving the clinical periodontal condition, improving the clinical presentation of RA and reducing the presence of periodontal pathogens. Thus, periodontal therapy should be strongly recommended for individuals with the concomitant presence of RA and PE.

DECLARATIONS

Ethical approval

The present controlled clinical trial was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE #03128012.0.0000.5149) and registered in the Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/).

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Conflict of Interest: The authors declare that they have no conflict of interest.

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TABLES

Table 1. Variabels of interest, periodontal clinical and microbiological parameters at T1.

Table 2. Periodontal condition of +PE+RA and +PE-RA groups at T1 and T2.

Table 3. Microbiological findings of +PE+RA and +PE-RA groups at T1 and T2.

Table 4. Correlations among bacterial counts and periodontal parameters in +PE+RA e +PE-RA groups.

FIGURES

Figure 1. Flow chart of the study design (CONSORT: Consolidated Standards of Reporting Trials).

Figure 2- The European League Against Rheumatism (EULAR) response criteria using the DAS-28.

Figure 3. Specific primers and probes used in the study

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Table 1. Variabels of interest, periodontal clinical and microbiological parameters at T1.

Variables	-PE-RA (n=30)	-PE+RA (n=23)	+PE+RA (n=24)	+PE-RA (n=30)	p
Sex [n (%)]					
Female	26 (66.7%)	20 (87%)	22 (91.7%)	20 (50%)	**<0.001***
Male	13 (33.3%)	3 (13%)	2 (8.3%)	20 (50%)	
Age (mean±s.d.)	45.3 ± 5.7	51.9 ± 11.3	54.1 ± 11.7	56 ± 8.1	**<0.001**†
DAS-28	NA	3.69 ±1.25	4.34 ± 0.89	NA	**0.024**†
Nº of teeth (mean±s.d.)	25.9 ± 2.3	21 ± 5.4	20.9 ± 5	24.3 ± 2.8	**<0.001**†
PD [n of sites (%)]					
< 3mm	3079 (83.6%)	1921 (99.4%)	2435 (65.4%)	1551 (77.4%)	
4-6mm	602 (16.3%)	11 (0.6%)	1166 (31.3%)	372 (18.6%)	**<0.001***
> 6mm	2 (0.1%)	0 (0.0%)	123 (3.3%)	81 (4.0%)	
PD (mean±s.d.)	2.7 ± 1.1	2.0 ± 0.7	3.4 ± 1.5	3.1 ± 1.6	**<0.001**†
CAL [n of sites (%)]					
< 3mm	3028 (82.8%)	1860 (96.3%)	2433 (64.1%)	1380 (68.9%)	
4-6mm	627 (17.1%)	70 (3.6%)	1100 (29.0%)	498 (24.9%)	**<0.001***
> 6mm	3 (0.1%)	2 (0.1%)	263 (6.9%)	126 (6.3%)	
CAL (mean±s.d.)	3.1 ± 0.9	2.2 ± 0.8	3.5 ± 1.8	2.4± 1.3	**<0.001**†
BOP (mean±s.d.)	0.37 ± 0.48	0.16 ± 0.37	0.47 ± 0.50	0.54 ± 0.50	**<0.001**†
*A. actinomycetemcomitans*3	76.4 ± 11.31	65.69 ± 14.23	92.07 ± 20.88	82.92 ± 11.26	**<0.001**†
*P. gingivalis*3	1.84 ± 0.62	2.12 ± 0.8	17.89 ± 4.76	20.30 ± 16.91	**0.0012**†
*T. dentícola*3	16.6 ± 8.7	12.2 ± 2.5	19.21 ± 6.07	16.78 ± 5.25	**0.010**†
*T. forsythia*3	31.1 ± 13.8	41.06 ± 18.5	59.51 ± 19.11	51.47 ± 20.19	**<0.001**†

*Chi square test; † Kruskal-Wallis test. All parwise comparisons through the Nemenyi post hoc test were significant (p<0.005), except from: PD (+PE+RA versus +PE-RA), CAL (-PE-RA versus-PE+RA), age (+PE+RA versus +PE-RA), number of teeth (-PE+RA versus +PE+RA), *A. actinonomycetemcomitans* (+PE-RA versus +PE-RA, -PE+RA versus +PE+RA), *P. gingivalis* (-PE-RA with all other groups, +PE+RA versus +PE-RA), *T. denticola* (-PE+RA versus +PE+RA, +PE+RA versus +PE-RA) and *T. forsythia* (-PE-RA versus –P+A, -PE+RA versus +PE+RA e +PE+RA versus+PE-RA); 3 bacterials counts (x10³) (mean±s.d.);

Table 2. Periodontal condition of +PE+RA and +PE-RA groups at T1 and T2.

Variables	+PE-RA (n=30)			+PE+RA (n=24)			T1 +PE+RA *versus* +PE-RA	T2 +PE-RA *versus* +PE-RA
	T1	T2	P	T1	T2	p		
PD [n of sites (%)]								
< 3mm	1551 (77.4%)	1944 (97.6%)		2435 (65.4%)	3125 (86.4%)			
4-6mm	372 (18.6%)	41 (2.1%)	**<0.001***	1166 (31.3%)	470 (13.0%)	**<0.001***	**<0.001***	**<0.001†**
> 6mm	81 (4.0%)	7 (0.4%)		123 (3.3%)	21 (0.6%)			
PD (mean±s.d.)	3.1 ± 1.6	2.0 ± 0.9	**<0.001†**	3.4 ± 1.5	2.5 ± 0.8	**<0.001†**	0.101#	**<0.001#**
CAL [n of sites (%)]								
< 3mm	1380 (68.9%)	1780 (88.8%)		2433 (64.1%)	3285 (88.7%)			
4-6mm	498 (24.9%)	189 (9.4%)	**<0.001***	1100 (29.0%)	274 (7.4%)	**<0.001**	**<0.001***	**<0.001***
> 6mm	126 (6.3%)	35 (1.7%)		263 (6.9%)	144(3.9%)			
CAL (mean±s.d.)	2.4 ± 1.3	2.5 ± 1.6	**<0.001†**	3.5 ± 1.8	2.4± 1.3	**<0.001†**	**<0.001#**	**0.005#**
BOP (mean±s.d.)	0.54 ± 0.50	0.10 ± 0.33	**<0.001†**	0.47 ± 0.50	0.15 ± 0.70	**<0.001†**	**<0.038#**	**<0.001#**

*Chi squared test; †Wilcoxon test; #Mann-Whitney test.

Table 3. **Table 3. Microbiological findings of +PE+RA and +PE-RA groups at T1 and T2.**

Bactéria (X10^3) mean ±s.d.	+RA+PE (N = 24)			+PE-RA (N = 30)			
	T1	T2	p*	T1	T2	P*	Intergroup p†
A.actinomycetemcomitans	92.07 ± 20.88	71.02 ± 23.04	**0.001**	82.92 ± 11.26	57.42 ± 15.42	**<0.001**	**0.020**
P. gingivalis	17.89 ± 4.76	11.07 ± 10.98	**0.008**	20.30 ± 16.91	14.1 ± 10.92	**0.021**	**<0.001**
T. denticola	19.21 ± 6.07	6.61± 2.34	**<0.001**	16.78 ± 5.25	7.89 ± 4.21	**<0.001**	0.35
T. forsythia	59.51 ± 19.11	26.43 ± 9.41	**0.001**	51.47 ± 20.19	29.42 ± 8.89	**<0.001**	0.81

*Wilcoxon test, ; †Mann-Whitney test.

Table 4. Correlations among bacterial counts and periodontal parameters in +PE+RA e +PE-RA groups.

Study groups	Periodontal parameters	*A. Actinomycetemcomitans*		*P. gingivalis*		*T. dentícola*		*T. forsythia*	
		r*	P	r*	p	r*	p	r*	p
+PE+RA (n=24)	**PD ≥ 4mm**	0.05	**<0.001**	0.09	**<0.001**	0.09	**<0.001**	0.14	**0.032**
	CAL ≥ 4mm	0.30	**0.021**	0.05	**<0.001**	0.09	**0.032**	0.04	**0.029**
	BOP	0.06	**<0.018**	0.08	**<0.001**	0.08	**<0.011**	0.10	**0.023**
+PE-RA (n=30)	**PD ≥ 4mm**	0.23	**<0.001**	0.04	**<0.001**	0.21	**0.032**	0.24	**<0.001**
	CAL ≥ 4mm	0.12	**0.031**	0.07	**0.021**	0.05	**0.041**	0.02	**0.004**
	BOP	0.07	**<0.001**	0.09	**0.015**	0.03	**0.036**	0.09	**0.017**

*Spearman correlation coefficient.

Figure 1. Flow chart of the study design (CONSORT: Consolidated Standards of Reporting Trials)

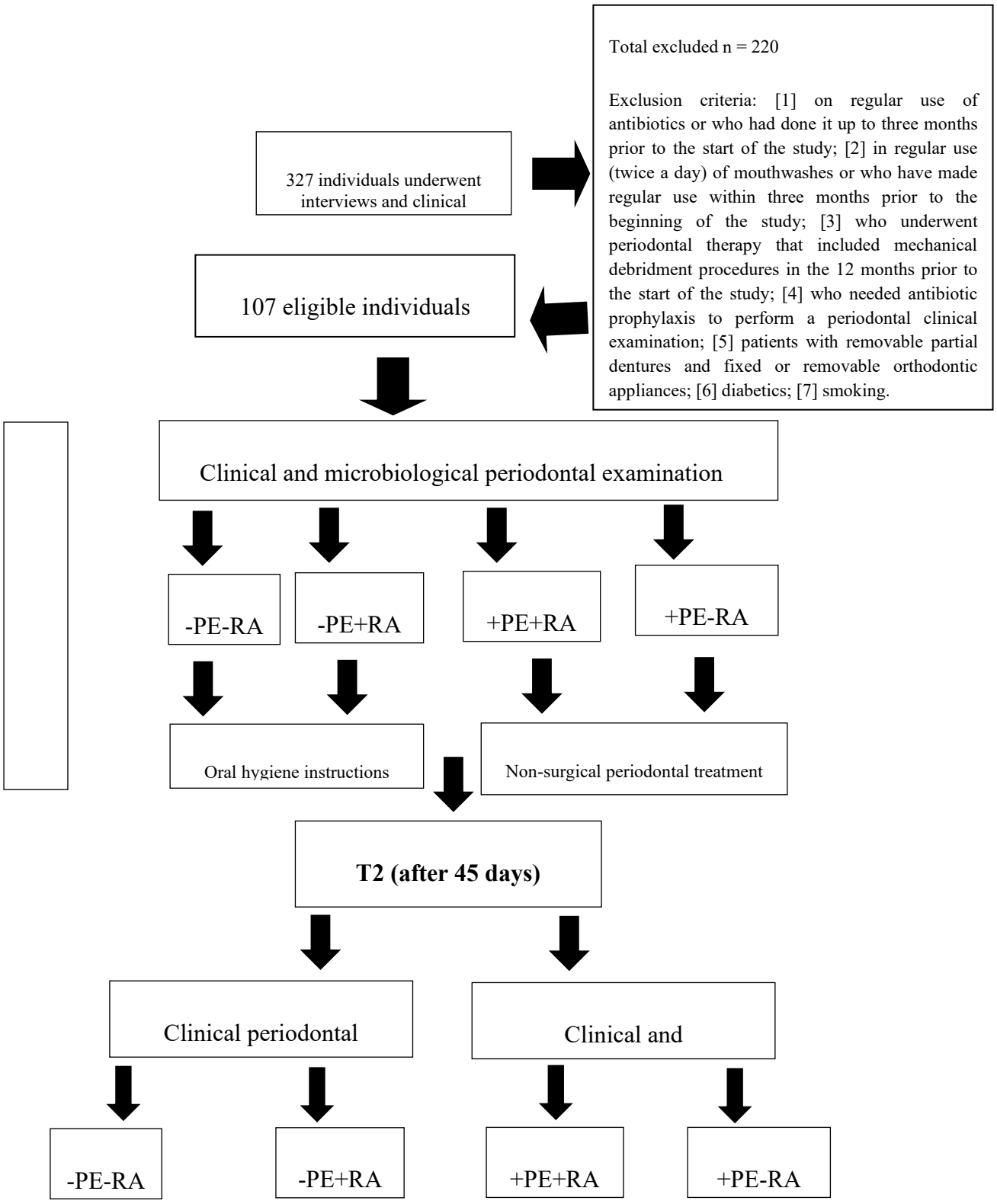

Figure 2. The European League Against Rheumatism (EULAR) response criteria using the DAS-28.

Current DAS28	Reduction of DAS28		
	>1.2	>0.6	≤1.2 ≤0.6
≤3.2	Good	Moderate	none
3.2 ≤ 5.1	Moderate	Moderate	none
>5.1	Moderate	None	none

Figure 3- Specific primers and probes used in the study

Microrganism	Primers
P. gingivalis	For: ACCTTACCCGGGATTGAAATG
	Rev: CAACCATGCAGCACCTACATAGAA
	Probe: TGACTGATGGTGAAAACCGTCTTCCCTTC
T. forsythia	For: AGCGATGGTAGCAATACCTGTC
	Rev: TTCGCCGGGTTATCCCTC
	Probe: CACGGGTGAGTAACG
T. denticola	For: CCGAATGTGCTCATTTACATAAAGGT
	Rev: GATACCCATCGTTGCCTTGGT
	Probe: ATGGGCCCGCGTCCCATTAGC
A. actinomycetemcomitans	For: GCGGCCAAAGTTTTTTCTTTTTCTT
	Rev: GCAATCCGTTTTCTTTAATTGATTTACG
	Probe: CCGGATTGGGACTAATT
Universal	For: TGGAGCATGTGGTTTAATTCGA
	Rev: TGCGGGACTTAACCCAACA
	Probe: CACGAGCTGACGACAAGCCATGCA

ARTIGO 3

NONSURGICAL PERIODONTAL THERAPY DECREASES THE SEVERITY OF RHEUMATOID ARTHRITIS AND THE PLASMATIC AND SALIVARY LEVELS OF RANKL AND SURVIVIN: A SHORT-TERM CLINICAL STUDY

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Authors contribution: All authors have made substantial contributions to the conception and design of the study. MFM, TAS, SRO, FQC, JRC and FOC

have been involved in data collection and data analysis. MFM, TAS, SRO, FQC, GAF, LOMC, SCC, JRC and FOC have been involved in data interpretation, drafting the manuscript and critically revising it, having given their final approval on the version to be published.

ABSTRACT

Aim: To investigate the influence of nonsurgical periodontal treatment (NSPT) on clinical periodontal status, RA activity and plasmatic and salivary levels of biomarkers through a controlled clinical trial on individuals with RA and periodontitis (PE).

Methods: Sixty-six individuals from a convenience sample were considered eligible and consecutively allocated in 3 groups: 1) individuals without PE and RA (-PE-RA, n=19); 2) individuals without PE and with RA (-PE+RA, n=23) and 3) individuals with PE and RA (+PE+RA, n=24). Full-mouth periodontal clinical examinations, Disease Activity Score (DAS-28) evaluations, and analysis in plasma and saliva of RANKL, OPG, RANKL / OPG and Survivin were performed at baseline (T1) and 45 days after NSPT (T2).

Results: NSPT in the +PE+RA group was very effective in improve periodontal condition. At T2, significant reductions in DAS-28 were observed in +PE+RA ($p=0.011$). Significantly higher levels of Survivin and RANKL were observed in saliva and plasma from RA individuals (with and without PE) compared to controls. Additionally, Survivin e RANKL demonstrated positive correlations with DAS-28 and an expressively significant reduction in +PE+RA at T2 ($p<0.001$).

Conclusions: NSPT was effective on improving both the periodontal and the RA clinical status and reducing the concentration of Survivin and RANKL in saliva and plasma.

Practical implications: Non-surgical periodontal treatment was effective reducing the concentration of Survivin and RANKL and on improving both the periodontal and the RA clinical status of affected individuals.

Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/).

Key words: clinical trials; nonsurgical periodontal debridements; periodontitis; rheumatoid arthritis; Biological markers.

INTRODUCTION

Periodontitis (PE) is a biofilm-induced chronic inflammatory disease that causes damage to the supporting tooth tissues. Its etiology is related to a polymicrobial synergy and dysbiosis model, inducing a non-resolving and tissue destructive host response, modulated by systemic diseases and genetic and environmental factors [1,2].

Rheumatoid arthritis (RA) is a chronic autoimmune disease that causes a breakdown of self-tolerance, chronic inflammation and debilitating joint destruction [3]. It is characterized by the presence of the rheumatoid factor (RF) and anti-protein / citrullinated peptide antibodies (ACPAs) related to disease activity [4].

Moreover, both are chronic inflammatory diseases characterized by an imbalance between the levels of pro-inflammatory and anti-inflammatory mediators such as interleukin (IL) -1, IL-6, IL-10, IL-11, IL-17, prostaglandin E2, the receptor activator of nuclear factor-kappa B ligand (RANKL) and the tumor necrosis factor (TNF)-α, resulting in bone loss [5-7]. Additionally, it has been suggested that the action of periodontal pathogens as resources of repeated transient systemic bacteremia might increase susceptibility to diabetes and RA through the metastatic spread of inflammation [4,8].

The understanding of the molecular mechanisms of metabolism and bone remodeling allowed the identification of RANKL molecules and their osteoprotegerin (OPG) ligand receptors, as well as members of the tumor necrosis factor family, as the main regulators of bone homeostasis both in health and disease [9,10]. These are involved in the pathophysiological mechanisms of bone loss in several diseases, such as RA [11,12] and PE [9,13,14].

Survivin is a multifunctional protein encoded by the BIRC5 gene, a member of the apoptostis inhibitor family [15-16] and it has been widely investigated in RA [15-19]. However, the role of Survivin in PE etiopathogenesis is still poorly studied [2,20,21]. Higher levels of this protein were found in gingival tissue samples from individuals with PE [20]. In addition, it has been shown that an important periodontal pathogen, Porphyromonas gingivalis, could promote apoptosis involving this mechanism in the PE etiopathogenesis, which

can be regulated by various anti- and pro-apoptotic molecules including Survivin [23]. Furthermore, apoptosis of higher Survivin expression has been demonstrated in the synovial tissue of individuals with RA showing a more erosive disease [15,17,19].

Concentrations of biomarkers relevant to the pathogenesis of diseases have been investigated in the oral and systemic spheres, using substrates such as saliva and blood. There is a great interest in saliva use in the diagnosis of oral and systemic pathologies, due to its easy collection and analysis [23]. However, it is controversial if the content of biomarkers in saliva and plasma are homologous and comparable [24,25].

In this scenario, it is hypothesized that non-surgical periodontal treatment (NSPT) reduces the salivary and plasma levels of RANKL, OPG, RANKL / OPG and Survivin, promoting an improvement in the clinical activity of RA and periodontal status.

The aim of this present study was to investigate the influence of NSPT on the clinical periodontal condition, the serological and salivary levels of the biomarkers RANKL, OPG, RANKL / OPG and Survivin on RA activity through a controlled clinical trial; as well as to compare whether these biomarkers have similar levels in both saliva and plasma.

METHODOLOGY

Study design and ethical issues

The present non-randomized clinical trial was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE #03128012.0.0000.5149) and registered in the Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8. The report of this study conformed the CONSORT checklist and statement. The present study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

Study sample and sampling strategy

Individuals with PE and RA diagnosis from the waiting list of the Rheumatology Service of the Hospital das Clínicas of the Federal University of Minas Gerais were invited to participate in the present study. Individuals without any rheumatological and periodontal diseases, randomly selected among accompanying relatives or companions, or employees of the respective reference centers and presenting same socioeconomic and cultural characteristics of cases were used as controls. They were invited to participate according to their accessibility and availability during cases recruitment routine. Using this approach, individuals were added to the study groups along the time of data collection until sample size was reached.

Sample size calculation was based on the data of Biyikoglu et al. [26] and Cosgarea et al. [27] considering a reduction of C-Reactive Protein [(CRP) values used in the calculation of the disease activity scores (DAS) index according to Prevoo et al. [28] by 50%, an alpha of 0.05 with a power of 80%, thus requiring 15 RA patients. Considering possible dropouts, at least 18 individuals per study group were recruited.

However, after adopting the exclusion criteria (Figure 1), 66 non-smokers and non-diabetics individuals were considered eligible from a convenience sample of 293 individuals that were interviewed and examined from September 2018 to January 2020, and consecutively included until the compounding of the following 3 groups: group 1 (control) – individuals without PE and RA (-PE-RA, n=19); group 2 – individuals without PE and with RA (-PE+RA, n=23); and group 3 – individuals with PE and RA (+PE+RA, n=24). Plus, an additional margin of 20% in the sample number per group was added due to the risk of future losses from follow-up exams.

Interviews were conducted by applying a standardized questionnaire aiming to collect the following data: sex, age, general health conditions, use of distinct disease modifying antirheumatic drugs [(DMARDS; particularly methotrexate and leflunomide), prednisone, vitamin D, calcium, and folic acid] and oral hygiene habits. Data on RA activity was also collected from participants' medical records by the Disease Activity Score (DAS-28) method [28].

After collecting these data, all individuals underwent a full-mouth periodontal examination, keeping records of periodontal clinical parameters,

evaluated in all teeth at six sites (mesio-buccal, disto-buccal, mid-buccal, mesio-lingual, disto-lingual, and mid-lingual): (1) probing depth (PD); (2) clinical attachment level (CAL); (3) gingival recession (REC), and (4) bleeding on probing (BOP). A manual periodontal probe (UNC-15 (PCPUNC-15, Hu-Friedy, Chicago, IL, USA), clinical mirror and gauze were used.

Additionally, periodontitis severity and activity were measured using the periodontal inflamed surface area (PISA) [29]. PISA reflects the surface area of bleeding pocket epithelium in mm2. PISA was calculated with a Microsoft Excel spreadsheet in the following steps: (a) Mean CAL and REC for each particular tooth is calculated; (b) Linear mean CAL and REC is translated into the periodontal epithelial surface area (PESA) for each specific tooth [30]. The PESA for a particular tooth consists of the root surface area of that tooth measured in mm2, which is covered with pocket epithelium; (c) The PESA for a specific tooth is then multiplied by the proportion of sites around the tooth that was affected by BOP, resulting in the PISA for that particular tooth; and (d) The sum of all individual PISAs around individual tooth is calculated, rendering the full- mouth PISA value in mm2 of each participant.

Periodontitis definition and staging

Individuals were defined as periodontitis cases and included in the sample if presenting Stage II (those with at least □2 interproximal sites with CAL of 3 to 4mm, with PD □5mm, with a pattern of horizontal bone loss up to the coronal third (15% to 33%) and without tooth loss due to periodontitis according to the criteria defined by Tonetti et al [31] or Stage III (those with ≥ 2 interproximal sites with CAL of ≥5mm, PD ≥6mm, vertical bone loss (≥3mm), with extension from the middle third to the apical root, with tooth loss due to periodontitis of up to 4 teeth, involvement of class II or III furcation) periodontitis. Individuals classified as Stage IV were excluded from the study.

Reumathoid arthritis diagnosis

The diagnosis and severity of RA were established by a group of rheumatologists associated with the Rheumatology Service of the Hospital das

Clínicas of the Federal University of Minas Gerais according to criteria of the American Society of Rheumatology [32]. RA can be classified as early RA (≤ 24 months of symptoms) or established RA (more than 24 months of symptoms), RA activity was assessed by using the DAS-28 index [28] in stages of activity, i.e., remission, mild, moderate and severe activity (according to the following DAS 28 cut-off points: remission (≤2.4), low (>2.4-3.6), moderate (>3.6-5.5), and high (>5.5) [32]. The DAS-28 evaluation included 28 joint counts added to the erythrocyte sedimentation rate or CRP and a general health assessment (VAS). Index calculation was performed in an application available at http:/www.4s-dawn.com/DAS28/. Only individuals with established RA and moderate disease activity (DAS 28 cut-off-points> 3.5-5-5) were included in the study. After therapeutic procedures, reductions in DAS-28 scores ≥ 1.2 are considered good performance [32].

Nonsurgical periodontal therapy (NSPT)

Individuals in the -PE-RA and -PE+RA groups (individuals without periodontitis) were handed toothbrushes and instructions for proper oral hygiene after periodontal examinations.

In the +PE+RA, scaling and root planing procedures were performed using a full-mouth disinfection (FMD) technique in a single stage (24 hours) divided into two sessions (60 min. per session) on two consecutive days, with the inclusion of chlorhexidine gel (CX) irrigation (1%) in the periodontal pockets after mechanical debridement, brushing of the tongue for 1 min. with CX gel (1%), and mouthwash at the beginning and end of each session with 0.12% CX for 30 seconds (gargle form in the last 10 seconds) [33]. Antibiotic therapy was not used as an adjunct to FMD procedures. It is also important to state that no tooth extraction was needed in the non-surgical periodontal therapy.

Periodontal clinical examinations and plasmatic and salivary analyses were performed at T1 (baseline) for the 3 groups. After 45 days (T2), new periodontal clinical examinations were performed for all groups; however, the plasmatic and salivary analysis was only performed again in +PE+RA group. DAS-28 was collected again after 45 days for the +PE+RA group. A flowchart with the study design is shown in Figure 1.

Initially, 15 individuals with periodontitis were selected for training and adaptation of study protocols. Periodontal examinations (PD and CAL records) were repeated in 5 individuals for each examiner (MFM, FOC and JRC) obtaining intra-examiner agreement. Weighted kappa agreement tests for clinical parameters of interest (PD and CAL) revealed intra and inter-examiner values greater than 92%. Data from this pilot study was not included in the final study. Examinations and treatment by FMD were performed by the same 3 properly trained examiners. When the exam was performed by one of these examiners, treatment and the second assessment was performed by the two others.

FMD procedures were performed by using Gracey and McCall curettes (Hu-Friedy, Chicago, IL, USA). An individual set of curettes was used for each group, being sharpened with each use and discarded after six consecutive uses.

Biomarkers quantification: saliva and plasma sampling

Paired samples of saliva and plasma were collected from the participants for the quantification of biomarkers. Individuals were asked to not eat or drink for at least one hour before saliva collection. Unstimulated saliva was collected using the spitting method, through which the participants were instructed to spit in a sterile collection tube for five minutes [34]. Then, all samples were centrifuged 350 × g for 10 minutes, diluted (1:1) in a phosphate-buffered saline (PBS) solution containing protease inhibitors, divided into aliquots and stored at -80°C. The collection was always performed in the mornings.

Peripheral blood samples were collected in tubes containing 7.2 mg of K2EDTA (BD Vacutainer™, Franklin Lakes, NJ, USA). Samples were centrifuged at 450× g for 10 minutes at room temperature 30 minutes after their collection, allowing one to obtain plasma collection. After centrifugation, the plasma aliquots were stored at -80°C.

Quantification of RANKL, OPG and Survivin was performed using comercially available ELISA Kits reactions (DuoSet® ELISA Human TRANCE / RANK L / TNFSF11 Kit, DuoSet® ELISA Human Osteoprotegerin/ TNFRSF11B

and Quantikine®ELISA Kit Human Survivin Immunoassay- R&D Systems Minneapolis, MN, USA). Tests were performed according to the manufacturer's instructions.

Statistical analysis

Sample characterization data, periodontal clinical parameters, plasmatic and salivary exams, and DAS-28 scores were analyzed in the baseline (T1) for all groups and 45 days after treatment (T2) for +PE+RA using the Chi-square, Kruskal-Wallis, Mann-Whitney, McNemar and Wilcoxon tests, when appropriate. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Pairwise comparisons were performed using the Nemenyi post-hoc test when there was a significant difference between the means of at least two groups. Group comparisons adjusted for age and gender were performed through Generalized Linear models (GLM). Spearman's correlation test was performed to assess the correlation between biomarkers levels and DAS-28 scores. All analyzes were performed using the statistical software R (Software R originally created by Ross Ihaka & Robert Gentleman, University of Auckland, New Zealand - version 3.6.3).

RESULTS

Table 1 shows the demographic variables such as age and sex, reflecting some peculiarities on both diseases, such as an older age in the groups with PE and high frequency of female individuals in the groups with RA.

Regarding the periodontal parameters, comparisons of interest demonstrated that the +PE+RA group had higher mean values of PD, CAL and % of patients with PD 4-6mm (unadjusted and adjusted $p<0.001$), as well as measures of current periodontal inflammatory activity (PISA) than the other group. Interestingly, for studies of associations between periodontitis and other systemic diseases, the PISA reflects the surface area of bleeding pocket epithelium in square millimeters. The surface area of bleeding pocket epithelium quantifies the amount of inflamed periodontal tissue.

Additionally, a smaller number of teeth in individuals in the +PE+RA group (unadjusted and adjusted $p<0.001$) was observed. NSPT in the +PE+RA group was effective in significantly reducing PD, BOP, PISA and improving CAL (Table 2).

In relation to DAS-28 scores at T1, -PE+RA and +PE+RA groups presented values of 3.69 ± 1.25 and 4.34 ± 0.89, respectively, with significant differences between them (unadjusted $p=0.024$; adjusted $p=0.019$). At T2, a significant reduction was observed in +PE+RA individuals, with the scores reduced to 3.12 ± 0.71 ($p=0.011$). The change in DAS scores from T1 to T2 (1.22) in the +PE+RA group was determined to be moderate according to the EULAR criteria (Figure 2), revealing a beneficial effect of NSPT on serological levels (reduction in CRP levels) and symptoms of RA (Table 2).

Regarding the biomarkers, Survivin and RANKL showed significantly higher mean values in individuals with RA with and without periodontitis in relation to healthy individuals (unadjusted and adjusted $p<0.05$). It is noteworthy the significant reduction in plasma and saliva levels of Survivin and RANKL after NSPT. Survivin and OPG showed significant differences in all intragroup comparisons. Furthermore, individuals in the +RA+PE group had higher levels of all biomarkers than individuals in the -PE+RA group (table 3). Overall, comparisons of the quantification of biomarkers levels in saliva and plasma were not homologous, showing significantly higher values in plasma compared to saliva, with the exception of only RANKL in the +RA+PE group (Table 3).

Correlations between DAS-28 scores and biomarkers are presented in Table 4. In particular, RANKL and Survivin showed significant positive correlations with DAS-28, e.g, the higher the quantification of these biomarkers, both in saliva and in the plasma, the greater the tendency to higher DAS-28 scores and vice-versa. In addition, from T1 to T2 in the +RA+PE group, the correlation values decreased and remained positive and significant (Table 4).

All participants in the RA groups were diagnosed with established RA. Consequently, all RA individuals were taking some type of DMARDS medication (62.5% methotrexate, 31.3% leflunomide and 6.2% others). However, the use of specific medication such as methotrexate, leflunomide, prednisone, vitamin D, calcium and folic acid by individuals with RA showed no

significant differences regarding the occurrence or severity of PE ($p>0.05$; data not shown).

DISCUSSION

The present study aimed to evaluate the effect of NSPT on RA disease activity in association with changes of periodontal parameters and levels of biomarkers (RANKL, OPG, RANKL/OPG and Survivin) in plasma and saliva. The outcome of NSPT in +RA+PE patients was compared to those in systemically healthy RA patients.

Findings from this present study have shown a beneficial effect of NSPT in reducing RA symptomatology, demonstrated by the significant reduction in DAS-28 scores. This benefit was also extended to serological reduction of CRP. Furthermore, NSPT decreased the levels of RANKL and Survivin along with significant improvements in all periodontal clinical parameters among individuals with PE and RA.

Several studies have reported that periodontitis could be a putative risk factor for RA or worsen the clinical and serological condition [13,27,35-38]. This relationship could be caused by: i) the coexistence of common risk factors for both diseases, such as age, smoking, and sex; ii) a common immunoregulatory imbalance;6,7 iii) underlying shared genetic risk factors; and/or iv) the possibility that periodontopathogenic bacteria could contribute to the etiology of rheumatic diseases [6,36].

In this study, NSPT was performed through the FMD technique due to logistical reasons and in order to avoid absenteeism. Since the presentation of this technique by Quirynen et al. in 1995, several studies have shown clinical and microbiological advantages of this technique over conventional scaling and root planning [33]. However, in a recent review, Pockpa et al. [39] reported an absence of significant differences between these two types of treatment, both being effective and able to be individually chosen by either dentists or patients.

As expected, there was an improvement in all periodontal clinical parameters after NSPT. There were significant reductions in the mean values of PD, BOP and CAL, as well as significant reductions in the % of sites with PD 4-6 and >6mm, and PISA scores revealing the efficiency of NSPT. This was

corroborated by other studies assessing the effect of periodontal treatment in individuals with RA [6,27,40]. Additionally, it was observed in the +PE+RA group at T1 positive and strong correlations between DAS-28 with Survivina and RANKL, with a great decrease in T2.

Improvements in periodontal clinical outcomes followed by significant reduction in DAS-28 scores (1.2 reduction from T1 to T2 in +PE+RA group) indicate that NSPT could improve the clinical and serological status of RA. These findings have also been reported in previous studies [23,26,27,38,41].

In recent systematic review and meta-analysis, Hussain et al. [42] reported there is consistent evidence suggesting that PE is associated with worse RA clinical activity as assessed by DAS-28 scores.

Consistently with the improvement of periodontal parameters after NSPT, +PE+RA group showed significant reductions in RANKL and Survivin without significant differences for OPG and RANKL/OPG.

In this particular case of microbiological and inflammatory interactions between RA and PE, findings have provided evidence for a relationship between the presence of inflammation associated with periodontal pathogens and the development of RA [27,37,40,43]. Pathogens such as Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema dentícula, Prevotella intermedia, Parvimonas micra, Fusobacterium nucleatum, Campylobacter rectus), Eikenella nodatum, Eikenella corrodens and Capnocytophaga species (Cs) are sources of repeated transient systemic bacteremias, which can increase susceptibility to RA through the metastatic spread of inflammation [40]. Particularly, P. gingivalis, a common pathogen in PE, could citrullinate proteins / peptides, inducing the autoimmune response and susceptibility of individuals to RA. This could be due to antibodies against citrullinated proteins / peptides being a common serological finding in both RA and PE [44].

In multicellular organisms, homeostasis is maintained through a balance between the cell proliferation and its death. Physiological cell death occurs mainly by apoptosis. Recent evidence suggests that changes in cell survival contribute to the pathogenesis of several diseases, including cancer, viral infections, neurodegenerative disorders and autoimmune diseases [16,17].

There are few studies on the expression of Survivin in the etiopathogenesis of RA,15-19 as well as PE [19-21]. Higher levels of Survivin were found in gingival tissue samples from individuals with chronic periodontitis [20]. Added to this, it has been demonstrated that an important periodontal pathogen, P. gingivalis, can promote apoptosis via regulation of various anti and pro-apoptotic including Survivin [22]. Survivin may be induced in periodontal tissues by microbial and host factors, participating in the apoptotic mechanisms involved in the inflammatory process associated with the destruction of gingival tissue during periodontitis [45]. Also, Survivin is involved in the survival of osteoclasts [46]. The reduction of Survivin would then result in greater apoptosis of these cells and consequently in less bone resorption. This mechanism could be linked to the positive response after periodontal therapy. Alongside this hypothesis, increased expression of Survivin was associated with greater joint damage and worse disease progression [18,47].

Recently, the increase of Survivin levels in the preclinical phase of RA and its association with NSPTs has been identified as a predictor of RA, a biomarker of joint damage, having poor response to antirheumatic treatment [19].

To the best of our knowledge, this is the first study to assess Survivin levels in individuals with RA and PE, focusing on the effect of NSPT underlying the association between these 2 conditions. The observation that higher plasma (+RA-PE and +RA+PE) and salivary (+RA-PE) levels of Survivin were present in individuals with RA reinforces the findings from studies that present Survivin as a marker of joint damage of RA [17,18,19,47]. Thus, a reduction in Survivin after NSPT can be an excellent parameter of the clinical outcome in individuals with PE and RA.

RANKL and its ligand receptor OPG, members of the tumor necrosis factor (TNF) family, are one of the main regulators of osteoclast development and its functions. RANKL signaling activates a variety of signal pathways participating in bone homeostasis and pathological processes [12]. In PE, it was shown that RANKL is positively regulated, while OPG, a bone protector, is negatively regulated compared to periodontal health, resulting in an increased RANKL / OPG ratio. These markers can be detected in gingival tissues and biological fluids, including GCF, saliva and blood [13].

In RA, RANKL expression occurs in the synovial fibroblasts of the joints and is predominantly responsible for the formation of osteoclasts and joint erosions [12]. OPG, on the other hand, protects bone integrity by decreasing osteoclastogenesis, preventing the RANKL-RANK interaction. The nexus between T cell activation, production of TNF-α, RANKL and OPG / RANK system in RA provides an insight into the mechanism of major bone loss patterns in RA [11]. After establishing the key molecular structure for better understanding the bone physiology, the inhibition of RANKL function has created new and promising strategies for the treatment of bone diseases, the prevention of bone destruction and cartilage damage in individuals with osteoporosis and RA, as well as bone loss associated with PE [12,13]. However, our findings did not show a reduction in OPG levels and RANKL / OPG ratio in +RA+PE subjects after NSPT.

Thus, levels of cytokines and unbalanced apoptosis could influence the severity of PE and worsen changes in the clinical status of RA due to a possibly longer life of inflammatory cells, mainly neutrophils and macrophages, leading to more extensive and uncontrolled tissue destruction.

Findings are still controversial if the content of biomarkers in saliva and plasma are homologous and comparable [24,25,48]. In this present study, comparisons of the quantification of biomarkers levels in saliva and plasma were not homologous and showed significant differences for all groups, with the exception of RANKL in the +PE+RA group. Hence, further studies should be carried out to assess if measurements in saliva and plasma may offer a convenient method to compare and evaluate the role of biomarkers in oral and systemic compartments [24]. RANKL and Survivin are valuable biomarkers for RA and PE, but the selection of sample materials is crucial. Plasma appears to be more suitable for RA and saliva to aid in periodontal diagnosis.

It should be observed that the use DMARDS may influence some of the molecules under investigation. However, all RA individuals in the sample were taking some type of DMARDS in both RA groups and showed no significant association with the occurrence or severity of PE. Since the aim of the study was to analyze the effect of periodontal treatment on plasma and salivary levels, some limitations should also be pointed such as a short-term follow-up time, the absence of positive controls, and the plasmatic and salivary

analysis being performed in only one group after treatment. Some degree of selection bias (controls) may also be present, but minimized through the same source population. Longitudinal studies, as well as periodontal maintenance therapy studies, should be conducted to assess the long-term effect of periodontal therapy for managing RA patients.

There is a consensus on the need to reduce the total inflammatory load in individuals with RA. Currently, RA management is based on early diagnosis, rigorous treatment and regular monitoring, with remission of the disease being the ultimate goal of treatment. Thus, the control of periodontal infection in patients with RA and PE can be an important therapeutic tool.

The present study can be considered an important starting point for further investigations on the effect of periodontal treatment in individuals with RA and its correlation with clinical, serological and immunological parameters. Thus, new studies with different populations and designs should be conducted, in order to confirm these findings and solidify the robustness of the scientific evidence of the association between periodontitis and RA. Moreover, further studies focusing on molecular outcomes, especially citrullinated proteins, would better assess the biological link between periodontitis and rheumatoid arthritis.

CONCLUSIONS

Non-surgical periodontal treatment was effective on improving both the periodontal and the RA clinical status and reducing the concentration of Survivin and RANKL in saliva and plasma. Consequently, periodontal therapy should be strongly recommended for individuals with the concomitant presence of RA and PE.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors MFM, TAS, LOMC, SRO, GAF, FQC, JRC, SCC and FOC declare that they have no conflict of interest.

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Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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TABLES AND FIGURES LEGENDS

Table 1. Demographic data of study groups.

Table 2: Periodontal clinical parameters and DAS-28 scores.

Table 3. Salivary and plasmatic levels of RANKL, OPG, RANKL/OPG and Survivin.

Table 4. Correlation between DAS-28 and biomarkers in –PE+RA- and +PE+RA groups.

Figure 1. Flow chart of the study design.

Table 1. Demographic data of study groups.

Variables	**-PE-RA (n = 19)**	**-PE+RA (n = 23)**	**+PE+RA (n = 24)**	***p***
Sex n (%)				
Female	13 (68.4%)	20 (87.0%)	22 (91.7%)	0.108*
Male	6 (31.6%)	3 (13.0%)	2 (8.3%)	
Age (years; mean ± s.d)	40.2 ± 17.5	51.9 ± 11.3	54.1 ± 11.7	**0.012**†

*Chi square test; †Kruskal-Wallis test. Post-hoc Nemenyi (Significant multiple comparisons: **-**PE+RA versus +PE+RA for age; p= 0.0863).

Table 2: Periodontal clinical parameters and DAS-28 scores.

Variables	-PE-RA (n = 19)	-PE+RA (n = 23)	+PE+RA T1 (n = 24)	+PE+RA T2 (n = 24)	T1 *Unajusted p*	*T1 Adjusted p*¶	T2 *p*
PD [n of sites (%)]							
< 3 mm	1921 (99.4%)	1916 (96.6%)	2435 (65.4%)	3125 (86.4%)			
4-6 mm	11 (0.6%)	62 (3.1%)	1166 (31.3%)	470 (13.0%)	**<0.001***	**<0.001**	**<0.001**‡
> 6 mm	0 (0.0%)	5 (0.3%)	123 (3.3%)	21 (0.6%)			
PD [mm (mean ± s.d.)]	2.0 ± 0.7	2.3 ± 0.9	3.4 ± 1.5	2.5 ± 0.8	**<0.001**†	**<0.001**	**<0.001**ǀ
CAL [n of sites (%)]							
< 3 mm	1860 (96.3%)	1862 (93.9%)	2433 (64.1%)	3285 (88.7%)			
4-6 mm	70 (3.6%)	116 (5.8%)	1100 (29.0%)	274 (7.4%)	**<0.001***	**<0.001**	**<0.001**‡
> 6 mm	2 (0.1%)	6 (0.3%)	263 (6.9%)	144(3.9%)			
CAL [mm (mean ± s.d.)]	2.2 ± 0.8	2.4 ± 0.9	3.5 ± 1.8	2.4 ± 1.3	**<0.001**†	**<0.001**	**<0.001**ǀ
BOP (mean % ± s.d.)	16 ± 37	24 ± 63	47 ± 50	15 ± 70	**<0.001**†	**<0.001**	**<0.001**ǀ
PISA (mm^2 m, mean ± s.d)	178.3 ± 312.21	197.2± 352.10	1038.41 ± 1132.70	514.23 ± 376.37	**<0.001**†	**<0.001**	**<0.001**ǀ
Number of teeth (mean ± s.d.)	26.1 ± 2.9	21.0 ± 5.4	20.9 ± 5.0	20.9 ± 5.0	**<0.001**†	**<0.001**	NA
DAS-28 (mean ± s.d.)	NA	3.69 ± 1.25	4.34 ± 0.89	3.12 ± 0.71	**0.024**‡	**0.019**	**0.011**ǀ

*Chi square test; †Kruskal-Wallis test; ‡Mann-Whitney test, §McNemarTest, ǀWilcoxon Test. Post-hoc Nemenyi. [All significant multiple comparisons, with the exception at T2: -PE+RA versus +PE+RA for PD (p=0.274) and CAL (p=0.055)]. ¶Generalized linear models adjusted for age and gender. PISA: periodontal inflamed surface area. NA = not applicable.

Table 3. Salivary and plasmatic levels of RANKL, OPG, RANKL/OPG and Survivin.

Biomarkers	-PE-RA (n = 19)	-PE+RA (n = 23)	+PE+RA T1 (n = 24)	+PE+RA T2 (n = 24)	T1 *Unadjusted p*	T1 adjusted p¶	T2 *p*
RANKL*(pg/mL)							
Saliva	22.9 ± 14.1	27.4 ± 21.5	39.4 ± 11.3	19.8 ± 15.8	**0.008***	**0.001**	**0.001**‡
plasma	27.3 ± 18.2	36.5 ± 39.1	41.3 ± 31.0	26.1 ± 20.1	**0.012***	**0.041**	**0.047**‡
	p=0.275†	***p*=0.047**†	*p*=0.779†	*p*=0.233†			
OPG* (pg/mL)							
Saliva	1638.2 ± 828.3	1649.6 ± 1110	1877.4 ± 1154.1	1880.2 ± 1233.3	0.600*	0.739	0.823‡
plasma	2687.5 ± 787.9	2703.2 ± 919.4	3128.9 ± 925.9	3036.5 ± 885.9	0.169*	0.340	0.257‡
	***p*=0.001**†	***p*=0.001**†	***p*<0.001**†	***p*=0.001**†			
Survivin*							
(pg/mL)	0.3 ± 0.9	14.9 ± 34.5	36.7 ± 93	27.2 ± 73.6	**0.019***	**0.004**	**0.007**‡
Saliva	35.1 ± 8.5	60.8 ± 127.1	416 ± 945.1	530.9 ±1147.8	**<0.001***	**<0.01**	**<0.001**‡
plasma	***p*=0.001**†	***p*=0.001**†	***p*<0.001**†	***p*=0.003**†			
RANKL/OPG*							
Saliva	0.010 ± 0.013	0.030 ± 0.034	0.091 ± 0.009	0.017 ± 0.013	0.091*	0.938	0.572‡
plasma	0.013 ± 0.011	0.015 ± 0.014	0.014 ± 0.013	0.01 ± 0.011	0.876*	0.246	0.450‡
	***p*=0.001**†	***p*=0.037**†	***p*=0.001**†	***p*=0.003**†			

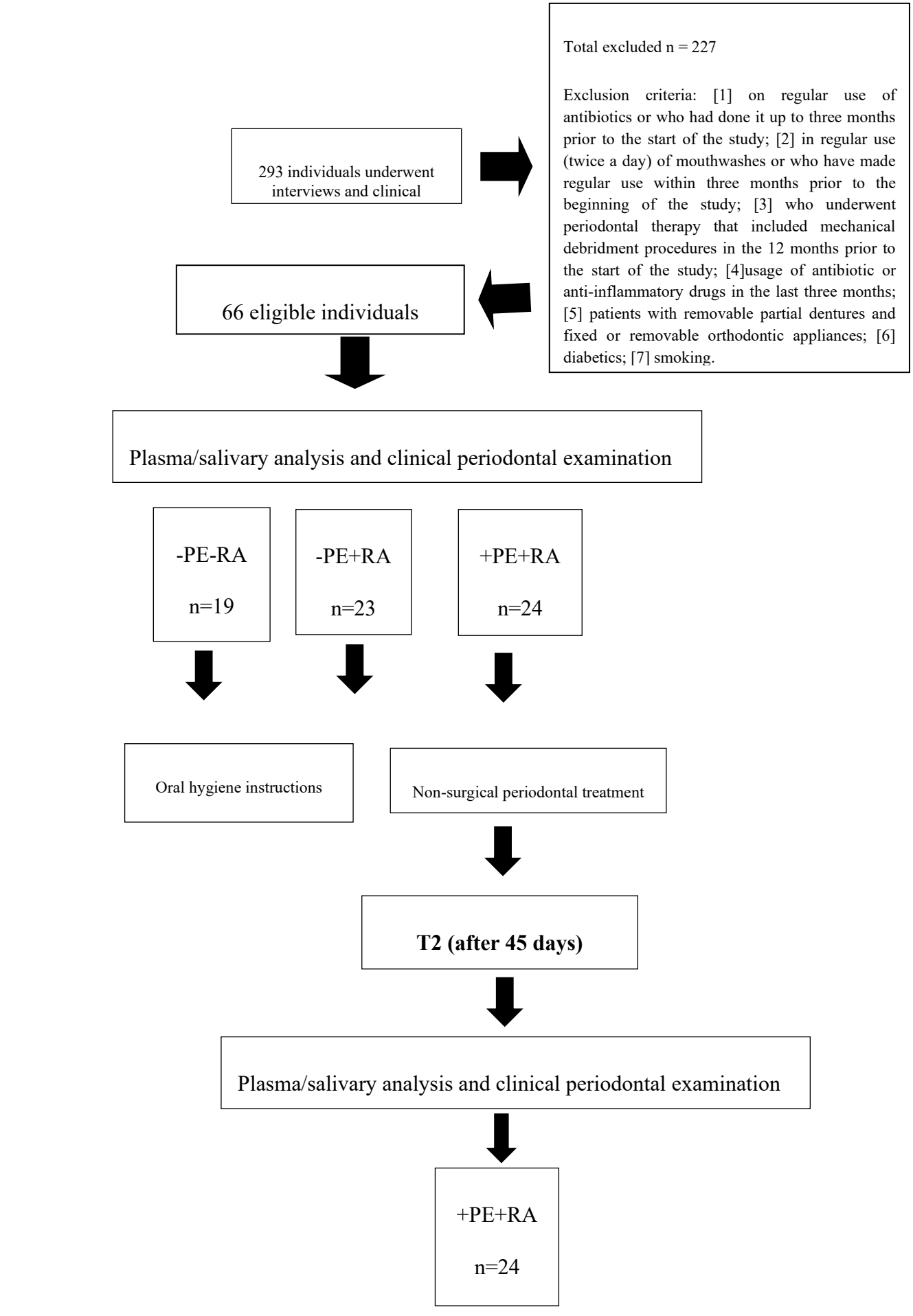
mean ± s.d. *Kruskal-Wallis test; †Mann-Whitney test, ‡ Wilcoxon test. Post-hoc Nemenyi [Significant multiple comparisons (p<0.05) for Survivin at T1 and T2. RANKL at T1: saliva and serum (-PE-RA versus-PE+RA) and T2 saliva e plasma -PE+RA versus +PE+RA]. ¶Generalized linear models adjusted for age and gender. Significant values in bold.

Table 4. Correlation between DAS-28 and biomarkers in -PE+RA and +PE+RA groups.

Biomarkers	-PE+RA (T1)	+PE+RA (T1)	+PE+RA (T2)
OPG			
saliva	-0.15	**-0.12**	-0.01
plasma	**-0.18**	**-0.05**	-0.01
RANKL			
saliva	**0.20**	**0.59**	**0.18**
plasma	**0.12**	**0.37**	**0.11**
Survivin			
saliva	**0.18**	**0.41**	**0.18**
plasma	**0.13**	**0.69**	**0.19**
RANKL/OPG			
saliva	-0.09	**-0.54**	-0.34
plasma	-0.03	**-0.09**	**-0.29**

Spearman correlations. Significant values in bold ($p<0.05$).

Figure 1. Flow chart of the study design



ARTIGO 4

ANTI-CARBAMYLATED PROTEIN ANTIBODIES AND CYCLIC CITRULINATED ANTI PEPTIDE ANTIBODIES IN INDIVIDUALS WITH RHEUMATOID ARTHRITIS AND PERIODONTITIS: A CONTROLLED CLINICAL TRIAL

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ABSTRACT

Introductions / Objective: Anti-protein / citrullinated peptide antibodies (ACPAs) and anti-carbamylated protein antibodies (Anti-CarP) levels are related to autoimmune diseases including rheumatoid arthritis (RA), but their association with periodontitis (PE) has been poorly studied and not yet elucidated. The aim this study was investigate the influence of non-surgical periodontal treatment (NSPT) on subgingival levels of *Porphyromonas gingivalis* and ACPAs and Anti-CarP through a pilot controlled clinical trial on individuals with RA and PE.
Methods: Twenty-six individuals were considered eligible and consecutively allocated in 3 groups: – individuals without RA and PE (RA-PE-, n=5, controls); – individuals with RA and without PE (RA+PE-, n= 9) and individuals with RA and PE (RA+PE+, n=12). Full-mouth periodontal clinical examinations, Disease Activity Score (DAS-28) evaluations, microbiological and ACPAs/Anti-CarP analysis were performed at baseline (T1) and 45 days after NSPT (T2).
Results: Significantly higher levels of ACPAs and Anti-CarP were observed in RA individuals (with and without PE) compared to controls. There was significant reduction in ACPAs (p=0.005) and Anti-CarP (p=0.032) in serum after NSPT in the RA+PE+ group. Positive and significant correlation values between DAS-28 and *P. gingivalis* and ACPAs/Anti-Carp in serum at T2were observed.
Conclusions: NSPT was effective on reducing the concentration of *P. gingivalis* and ACPAs/Anti-CarP in serum.

Keywords: biological markers; clinical trials; nonsurgical periodontal debridement; periodontitis; rheumatoid arthritis.

KEY-POINTS

Periodontal therapy was effective in reducing the subgingival levels of *P. gingivalis* and the concentrations of ACPAs and Anti-CarP antibodies with the improvement of periodontal and RA clinical parameters.

Periodontal therapy should be strongly recommended for individuals with the concomitant presence of RA and PE.

DECLARATIONS

Funding

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Conflicts of interest/Competing interests

The authors declare that there were no conflicts of interest.

Ethics approval

The present pilot controlled clinical trial was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE #03128012.0.0000.5149)

Consent to participate (include appropriate statements)

Consent for publication (include appropriate statements)

Availability of data and material

All of the underlying research material related to our article can be accessed on demand by e-mail notification.

Authors' contributions

All authors have made substantial contributions to the conception and design of the study. MFM, TAS, DCC, GAF, and FOC were involved in data collection and data analysis. MFM, TAS, SRO, DCC, GAF, LOMC, SCC, JRC, and FOC were involved in data interpretation, drafting the manuscript and revising it critically and have given final approval of the version to be published.

INTRODUCTION

Periodontitis (PE) is an oral disease characterized by a bacterial-induced inflammation. Its pathogenesis involves complex interactions between periodontal pathogens and host immune responses, resulting in the progressive loss of the attachment apparatus and bone around teeth. The abundance of inflammatory pathways in this pathobiological process has raised the attention to the systemic impacts of periodontitis [1].

RA is a chronic autoimmune disease that causes a breakdown of self-tolerance, chronic inflammation and debilitating joint destruction [2]. It is characterized by the presence of rheumatoid factor and citrullination (conversion of arginine residues into citrulline) with induction of anti-protein/citrullinated peptide antibodies (ACPAs), which are related to disease activity. It constitutes itself as a specific biomarker that can be detected years before the RA symptoms [3,4].

Several studies have reported that periodontitis could be a putative risk factor for RA or worsen clinical RA parameters [5,6,7,8,9]. These findings are supported by some similarities in pathophysiological pathways resulting from unregulated chronic inflammatory cascades, in addition to the environmental and genetic factors that play a crucial role, potentiating tissue destruction in both diseases [10,11, 12].

Carbamylation is another post-translational modification of proteins also producing autoantibodies that can trigger inflammatory conditions, including RA [13, 14]. Recent evidence has suggested that anti-carbamylated protein antibodies (Anti-CarP) may be useful as a diagnostic marker for RA, but are independent of ACPAs [3,4,15,16]. Experimental studies have also shown that the detection of Anti-CarP antibodies in the serum precedes the RA onset [17,18]. Bright *et al.* [19] reported that protein carbamylation was observed in PE as a result of chronic gingival inflammation, and thus would generate the production of Anti-CarP antibodies.

Therefore, there could be a link between the production of these antibodies in the association between RA and PE. Recently, the presence of

Anti-CarP has also been demonstrated in inflamed periodontal tissues in patients with mild to moderate periodontitis [19]. Circulating levels of carbamylated proteins have been associated with the severity of PE and influenced by periodontal treatment in patients with RA [4].

In addition to these factors of similarity between PE and RA, findings provided evidence for a relationship between the presence of inflammation associated with periodontal pathogens and the development of RA. Particularly, *Porphyromonas gingivalis*, a common pathogen in PE, could cause citrullination of proteins, triggering the production of ACPAs that is a common serological finding in both RA and PE [20]. Similarly, there was a positive and significant association between CarP and *P. gingivalis* in individuals with early RA [16].

Thus, protein carbamylation and ACPAs levels are related to autoimmune diseases including RA, but their association with PE has been poorly studied and not yet elucidated, therefore requiring further studies [4].

The aim of this present study was to investigate the influence of nonsurgical periodontal treatment (NSPT) on subgingival bacterial levels of *P. gingivalis* and concentration of ACPAs/Anti-CarP through a pilot controlled clinical trial on individuals with RA and PE.

MATERIALS AND METHODS

Study design and ethical issues

The present pilot controlled clinical trial was approved by the Research Ethics Committee of the Federal University of Minas Gerais (CAAE #03128012.0.0000.5149) and registered in the Brazilian Registry of Clinical Trials (ReBEC) protocol #RBR-8g2bc8 (http://www.ensaiosclinicos.gov.br/rg/RBR-8g2bc8/). The report of this study conformed the CONSORT checklist and statement. This study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013. Participants were informed about the research objectives and signed an informed consent form prior to entering the study.

Study sample and sampling strategy

Individuals with a diagnosis of PE from the waiting list of the School of Dentistry of the Federal University of Minas Gerais (UFMG) and with a diagnosis of RA from the Rheumatology Service of the University Hospital of UFMG were invited to participate in the present study. Individuals without any rheumatological and periodontal diseases, randomly selected among relatives, companions or employees of the respective reference centers were used as controls.

From a convenience sample of a previous study [21], 26 non-smokers and non-diabetics individuals were considered eligible and consecutively included until the compounding of the following 3 groups: group 1 (controls) – individuals without RA and PE (RA-PE-, n=5); group 2 – individuals with RA and without PE (RA+PE-, n= 9); group 3 – individuals with RA and PE (RA+PE+, n=12).

Interviews were conducted by applying a standardized questionnaire aiming to collect the following data: gender, age, general health conditions, use of distinct medications (particularly methotrexate, vitamin D, calcium, folic acid, leflunomide and prednisone) and oral hygiene habits. Data on RA activity was also collected from participants' medical records by the Disease Activity Score (DAS-28) method [22]. (Prevoo *et al,* 1995).

After collecting these data, all individuals underwent a full-mouth periodontal examination, keeping records of periodontal clinical parameters, which were evaluated in all teeth at four sites (mesial, distal, buccal and lingual): (1) probing depth (PD); (2) clinical attachment level (CAL); (3) bleeding on probing (BOP). A manual periodontal probe (UNC-15 (PCPUNC-15, Hu-Friedy, Chicago, IL, USA), clinical mirror and gauze were used.

Periodontitis definition and staging

Individuals were defined as being periodontitis cases and classified by staging from Stage II if presenting at least ≤2 interproximal sites with CAL of 3 to 4mm, with PD ≥5mm, with a pattern of horizontal bone loss up to the coronal third (15% to 33%) and without tooth loss due to periodontitis according to the criteria defined by Tonetti, et al., [23].

Reumathoid arthritis diagnosis

The diagnosis and severity of RA (mild, moderate or severe forms) were established by certified rheumatologists (D.C.C and G.A.F) according to criteria of the American Society of Rheumatology [24]. Additionally, RA activity was assessed by using the DAS-28 method [22] in stages of activity, i.e., remission, mild, moderate and severe activity. After therapeutic procedures, reductions in DAS-28 scores ≥1.2 were considered good performances.

Nonsurgical periodontal therapy (NSPT)

Individuals in the RA-PE- and RA+PE- groups (individuals without periodontitis) were handed toothbrushes and instructions for proper oral hygiene after periodontal examinations.

In the RA+PE+, scaling and root planing procedures were performed using a full-mouth disinfection (FMD) technique in a single stage (24 hours), divided into two sessions (60 min. per session) on two consecutive days, with the inclusion of chlorhexidine gel (CX) irrigation (1%) in the periodontal pockets after mechanical debridement, tongue brushing for 1 min. with CX gel (1%), and mouthwash at the beginning and end of each session with 0.12% CX for 30 seconds (gargle form in the last 10 seconds) [25]. Antibiotic therapy was not used as an adjunct to FMD procedures.

Periodontal clinical examinations, microbiology and serum/salivary analysis were performed at T1 (baseline) for the 3 groups. After 45 days (T2), new periodontal clinical examinations were performed for all groups. However, the serum/salivary analysis was only performed again in the RA+PE+ group, and DAS-28 being collected again after 45 days.

Examinations and treatment by FMD were performed by 3 trained and calibrated periodontists (MFM, FOC and JRC). When an exam was performed by one of these examiners, treatment and the second assessment was performed by the two others. Weighted kappa agreement tests for clinical parameters of interest (PD and CAL) revealed intra and inter-examiner values greater than 92%.

FMD procedures were performed by using Gracey and McCall curettes (Hu-Friedy, Chicago, IL, USA.). An independent set of scrapers was used for each group, being sharpened with each use and discarded after six consecutive uses.

Microbiological analysis

Subgingival plaque samples were collected as described in our previous study [26]. Supragingival dental plaque was removed using a sterile curette, and a sterile fine paper point (Johnson & Johnson, New Brunswick, NJ, USA) was inserted into the gingival sulcus/periodontal pocket and kept there for 10 seconds. Then, the paper point was placed in a vial containing 1.0 ml of phosphate-buffered saline (pH7.4), inserted in a minitube and kept on ice. A vortex mixer at maximum speed was used for 1 min and stored in a freezer at -80°C until further analyses to obtain bacterial dispersion.

The genomic DNA (gDNA) was extracted and purified using a commercial Genomic DNA Mini Kit (Life Technologies, Carlsbad, CA, USA), following the manufacturer's specifications. The total microbial count of *Porphyromonas gingivalis* was performed by quantitative real-time polymerase chain reaction (qPCR) using a set of TaqMan (Life Technology, Carlsbad, CA, USA) primers/probes in Real-Time PCR System, also following manufacturer's instructions (Primers and probes – *P. gingivalis*: For: ACCTTACCCGGGATTGAAATG; Rev. CAACCATGCAGCACCTACATAGAA; probe: TGACTGATGGTGAAAACCGTCTTCCCTTC). The qPCR conditions were: 50º C for 2 min, 95º C for 10 min, 40 cycles of 95º C for 15 s, and 60º C for 1 min.

Anti-CCP and anti-CarP antibodies analysis

Levels of anti-CCP in saliva and serum and anti-Carp in serum were measured at T1 and T2. Unstimulated saliva was obtained by spitting method for five minutes in sterile containers [27]. Peripheral blood samples were collected in appropriated tubes (BD Vacutainer ™, Franklin Lakes, NJ, USA). Anti-CCP and anti-CarP antibodies were quantified by using the commercially

available kits (Anti-CCP ELISA (IgG) kit, EUROIMMUN, Bernstadt auf dem Eigen, Saxony, Germany; Anti-CarP Ab ELISA kit, NOVATEINBIO, Hudson, MA, USA). The optical absorbance was measured with a microplate reader (Molecular Devices, San Jose, CA, USA).

Statistical analysis

Sample characterization data, periodontal clinical parameters, microbiological analysis, serum/salivary exams and DAS-28 scores were analyzed at baseline (T1) for all groups and 45 days after treatment (T2) for the RA+PE+ group using the Chi-square, Kruskal- Wallis, Mann-Whitney, McNemar, Wilcoxon tests when appropriate. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. When there was a significant difference between the means of at least two groups, pairwise comparisons were performed using the Nemenyi post-hoc test. Spearman's correlation test was performed to assess the correlation between biomarkers levels and DAS-28 and *P. gingivalis* levels. All analyzes were performed using the statistical software R (Software R originally created by Ross Ihaka & Robert Gentleman, University of Auckland, New Zealand (version 3.6.3).

RESULTS

Table 1 shows the demographic variables of study groups, such as age and gender, reflecting some peculiarities of both diseases. Older age in the groups with PE and high frequency of female individuals in the groups with RA was observed.

Regarding the periodontal parameters, comparisons of interest demonstrated that the RA+PE+ group had higher mean values of PD ($p<0.001$), CAL ($p<0.001$) and % of individuals with PD 4-6mm ($p<0.001$) than other groups. In addition, a smaller number of teeth in individuals in the RA+ PE+ group ($p=0.001$) was also observed. NSPT in the RA+PE+ group was effective in significantly reducing PD, BOP and improving CAL (Table 2).

In relation to DAS-28 scores at T1, RA+PE- and RA+PE+ groups presented values of 3.71± 1.24 and 4.44 ± 0.90, respectively, with significant

differences between them (p=0.024). At T2, a significant reduction was observed in RA+PE+ individuals, with the scores reduced to 3.10 ± 0.70 (p=0.011). The change in DAS scores from T1 to T2 (1.3) in the RA+PE+ group was determined to be good according to the EULAR criteria, revealing a beneficial effect of NSPT on serological levels (reduction in CRP levels) and symptoms of RA (Table 2).

Regarding the concentration of ACPAs in serum and saliva and Anti-CarP in serum, higher mean values in individuals with RA with and without periodontitis in relation to healthy individuals was observed, with significantly higher values of ACPAS in serum of individuals in the RA+PE+ group (p=0.034; Table 3). The significant reduction in ACPAs (p=0.005) and Anti-CarP (p=0.032) in serum after NSPT in RA+PE+ group (Table 4) is noteworthy.

Correlations between DAS-28 scores, *P. gingivalis* levels and biomarkers in RA+PE+ group are presented in Table 5. In particular, *P. gingivalis* levels and DAS-28 showed significant positive correlations with serum ACPAs and Anti-CarP, *i.e*, the higher the quantification of these biomarkers, both in saliva and in the serum, the greater the tendency to higher DAS-28 scores and *P. gingivalis* loads and vice-versa. Positive and significant correlation values between DAS-28 and *P. gingivalis* and ACPAs/Anti-Carp were also observed and decreased after NSPT (Table 5).

The use of specific medication such as methotrexate, vitamin D, calcium, folic acid, leflunomide and prednisone by individuals with RA showed no significant differences regarding the occurrence or severity of PE (p>0.05; data not shown).

DISCUSSION

In this present study, a significant reduction in serum levels of ACPAs and Anti-Carp antibodies after NSPT was demonstrated. The beneficial effect of NSPT in the main periodontal and RA clinical parameters was verified as well. Furthermore, a significant positive correlation was observed between ACPAs and Anti-Carp with *P. gingivalis* and DAS-28. These findings reinforce the evidence on the association between RA and PE and the positive impact of periodontal treatment in both diseases.

PE and RA share common characteristics and pathogenic similarities in relation to immunogenetics and tissue destruction pathways [28]. (Loutan *et al,* 2019). Both are chronic inflammatory diseases that share mutual risk factors for susceptibility, such as HLA-DRBI alleles and smoking. The role of *P. gingivalis* and its enzyme peptidyl arginine deiminase (PAD), that is capable of generating citrullinated epitopes that are recognized by ACPAs, has been proposed as a link between PE and RA. Recently, two isoforms of the peptidyl arginine deiminase family (PAD2 and PAD4) and citrullinated proteins have been identified in inflamed periodontal tissues, reinforcing the hypothesis that periodontal citrullination may play an important role in the etiopathogenesis of RA [28,29].

The systemic effect of NSPT in the clinical course of RA was previously demonstrated [30, 31,32,33], corroborating our results. A hypothesis that must be considered is that the reduction of periodontopathogens decrease local and systemic inflammation [34] and the exposure of joints to circulating bacterial virulence factors [30].

Another relevant point observed in this present study is the significant reduction in serum levels of ACPAs and anti-Carp after NSPT like the findings of Okada *et al. [32].* and Kaneko *et al. [4].* Citrullination and carbamylation are important sources of autoantibodies that play a role in the development and progression of RA. However, both processes can arise due to inflammatory reactions occurring outside the synovium [14,35]. It has been hypothesized that inflamed periodontium may be a source of autoantibodies production [19,36].

Interestingly, *Porphyromonas gingivalis,* a periodontitis-related bacterium, is the only known microorganism that produces Pg peptidyl arginine deiminase, an enzyme that causes citrullination of proteins [37,38]. This enzyme exhibits the same physiological activity as PAD of human origin [39,40]. These citrullinated proteins such as vimentin are the ones to arise autoantibody ACPAs. In a Nagahama cohort study in which approximately 10,000 healthy citizens who have not developed RA were monitored for 5 years. The significant associations between periodontal disease parameters and positivity and levels of ACPAs in healthy population support the fundamental involvement of periodontal disease with ACPA production [41].

A recent study reinforces the relevance of periodontal disease in RA. *Porphyromonas gingivalis* was administered into the oral cavity of mice and the amount of ACPAs in serum and saliva was measured using ELISA. Antibodies in serum, saliva, and joints were analyzed by western blotting. There was greater inflammatory cell infiltration in joints of mice with RA infected with *P. gingivalis* than those with RA alone. Furthermore, ELISA results showed that the amount of ACPAs in serum was significantly higher in the *P. gingivalis* and RA groups, with a tendency of increase in the saliva. Accordingly, western blotting results showed higher citrullinated protein in the serum and saliva in the RA and *P. gingivalis* and RA groups. It was concluded that *P. gingivalis* infection increases ACPAs in the serum and is reflected in the saliva. Hence, it may be involved in the inflammatory progression of RA [40].

In our experimental conditions, salivary levels of anti-CCP were lower than serum and no significant differences were seen in individuals with or without PE. Consistently, no changes were observed after NSPT. A previous study have detected anti-CCP in saliva of RA patients, but the contribution of periodontal status was not evaluated [42]. The meaning of anti-CCP in saliva and its relationship with serum levels and disease outcomes deserves further investigation.

Significant positive correlation between anti-*P. gingivalis* serum levels and anti-CCP antibodies was previously demonstrated, as well the decrease of both parameters after periodontal therapy [4, 30, 32]. This present study obtained a positive and significant correlation between DAS-28 and *P. gingivalis* and ACPAs and Anti-Carp antibodies. These data support a role for *P. gingivalis* in protein citrullination and carbamylation, and consequently in the production of anti-CCP and anti-CarP antibodies.

The current pilot study has some limitations. The sample size of each group can be considered small, so that the reliability of our study may be limited. Findings should be confirmed in future studies with samples comprising a larger number of individuals from different populatios. However, the results obtained are promising and relevant due to the limited literature background. This present study should be considered a starting point for future research.

In conclusion, NSPT was effective in reducing the subgingival levels of *P. gingivalis* and the concentrations of ACPAs and Anti-CarP antibodies in

serum along with the improvement of periodontal and RA clinical parameters. *P*. gingivalis and autoantibodies might be useful markers for monitoring individuals at risk for RA and PE.

CONFLICT DE INTEREST

The authors declare that there were no conflicts of interest.

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Table 1. Demographic data of study groups.

Variables	RA-PE- (n = 5)	RA+PE- (n = 9)	RA+PE+ (n = 12)	*p*
Gender n (%)				
Female	4 (80.0%)	9 (87.0%)	13 (91.7%)	0.108*
Male	1 (20.0%)	1 (13.0%)	1 (8.3%)	
Age (years; mean ± s.d.)	40.4 ± 17.2	52.1 ± 11.2	53.9 ± 11.2	**0.011****

*Chi square test; **Kruskal-Wallis test. Post-hoc Nemenyi test (significant multiple comparisons: RA+PE-versus RA+PE+ for age).

Table 2: Periodontal clinical parameters and DAS-28 scores of study groups.

Variables	RA-PE- (n = 5)	RA+PE- (n = 9)	RA+PE+ T1 (n = 12)	RA+PE+ T2 (n = 12)	T1 *p*	T2 *p*
PD [n of sites (%)]						
< 3 mm	405 (99.3%)	833 (96.1%)	1420 (65.4%)	1823 (86.4%)		
4-6 mm	3 (0.7%)	27 (3.1%)	680 (31.3%)	274 (13.0%)	<0.001*	<0.001****
> 6 mm	0 (0.0%)	2 (0.2%)	72 (3.3%)	12 (0.6%)		
PD (mean ± s.d.)	2.0 ± 0.5	2.2 ± 0.8	3.5 ± 16	2.6 ± 0.9	<0.001**	<0.001*****
CAL [n of sites (%)]						
< 3 mm	392 (96.3%)	810 (93.9%)	1420 (64.1%)	1916 (88.7%)		
4-6 mm	15 (3.6%)	50 (5.8%)	642 (29.0%)	160 (7.4%)	<0.001*	<0.001****
> 6 mm	1 (0.1%)	3 (0.3%)	153 (6.9%)	84 (3.9%)		
CAL (mean ± s.d.)	2.2 ± 0.6	2.3 ± 0.7	3.6 ± 1.8	2.5 ± 1.6	<0.001**	<0.001*****
BOP (mean ± s.d.)	0.14 ± 0.31	0.24 ± 0.63	0.49 ± 0.52	0.16 ± 0.88	<0.001**	<0.001*****
Number of teeth (mean ± s.d.)	26.3 ± 2.8	22.0 ± 5.8	20.7 ± 5.1	20.5 ± 5.0	<0.001**	NA
DAS-28 (mean ± s.d.)	NA	3.71 ± 1.24	4.44 ± 0.90	3.10 ± 0.70	0.024***	0.011******
P. gingivalis	1.81 ± 0.67	2.42 ± 0.9	19.41 ± 4.81	11.01 ± 10.12	<0.001***	<0.001*****

*Chi square test; **Kruskal-Wallis test; ***Mann-Whitney test, ****McNemarTest, *****Wilcoxon Test. Post-hoc Nemenyi test [all significant multiple comparisons, with the exception at T2: RA+PE- versus RA+PE+ for PD (p=0.286) and CAL (p=0.062)].NA=not applicable

Table 3. Anti-citrullinated protein antibodies (ACPAs) levels in serum and saliva and anti-carbamylated proteins antibodies (Anti-CarP) in serum at T1.

Variables	**RA-PE-** (n=5)	**RA+PE-** (n=9)	**RA+PE+** (n=12)	***p*** *
ACPAs serum [RU/mL (mean ± s.d.)]	22.93 ± 12.75	113.50 ± 71.39	117.20 ± 53.94	**0.034**
ACPA saliva [RU/mL (mean ± s.d.)]	9.36 ± 6.35	33.22 ± 21.53	28.30 ± 20.34	0.137
Anti-CarP serum [ng/mL (mean ± s.d.)]	NA	2.50 ± 2.41	2.83 ± 2.07	0.599

NA not analysed; *Mann-Whitney test

Table 4. Levels of anti-citrullinated protein antibodies (ACPA) and anti-carbamylated proteins antibodies (Anti-CarP) of individuals with established rheumatoid arthritis (RA) before and after periodontal therapy.

Variables	**RA+PE+ (n=12)**		
	T1	**T2**	***p*** *
ACPAs serum [RU/mL (mean ± s.d.)]	117.20 ± 53.94	98.53 ± 67.04	0.005
ACPAs saliva [RU/mL (mean ± s.d.)]	28.30 ± 20.34	27.51 ± 26.88	0.784
Anti-CarP serum [ng/mL (mean ± s.d.)]	2.83 ± 2.07	0.98 ± 1.67	0.032

*Wilcoxon Test.; s.d.: standard deviation.

Table 5. Correlations between DAS-28 scores and *P. gingivalis* levels and biomarkers in the RA+PE+ group.

Biomarkers	RA+PE+ group (n = 12)	
	DAS-28	
	T1	T2
ACPA (RU/mL)		
serum	**0.69**	**0.15**
saliva	-0.12	0.11
Anti-CarP (ng/mL		
serum	**0.52**	**0.21**
	P. gingivalis	
	T1	T2
ACPA (RU/mL)		
serum	**0.52**	**0.23**
saliva	0.22	0.17
Anti-CarP (ng/mL		
serum	**0.48**	**0.26**

Spearman correlation. Significant values are presented in bold ($p<0.05$).

5 – CONSIDERAÇÕES FINAIS

O presente estudo reportou uma alta prevalência de PE em indivíduos com AR e uma importante associação entre ocorrência, gravidade e extensão da PE associadas à AR e ao tabagismo. O tratamento periodontal não cirúrgico foi eficaz em melhorar a condição cliníca periodontal, reduziu a presença de patógenos periodontais (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia, Treponema denticola*) e melhorou o quadro clínico da AR (redução dos escores de DAS-28). Adicionalmente, reduziu ainda os níveis de survivina, RANKL e concentração de ACPAs e Anti-CarP. Neste sentido, nossos achados corroboram a robustez das evidências científicas da associação entre periodontite e AR.

Neste cenário, é consenso a necessidade de redução da carga inflamatória total em indivíduos com AR. Atualmente, o manejo da AR é baseado no diagnóstico precoce, tratamento rigoroso e monitoramento regular, sendo a remissão da doença o objetivo final do tratamento. Assim, o controle da infecção periodontal em pacientes com AR e PE pode ser uma importante ferramenta terapêutica.

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ANEXO - Termo de consentimento livre e esclarecido

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: DOENÇA PERIODONTAL E DOENÇAS REUMÁTICAS: AVALIAÇÃO DE ASSOCIAÇÕES CLÍNICAS, IMUNOLÓGICAS, GENÉTICAS E MICROBIOLÓGICAS Subprojeto: Artrite Reumatóide e Doença Periodontal: Associação entre Tratamento periodontal, Microbioma oral e Terapia com Modificadores do curso da doença.

Pesquisador: Gilda Aparecida Ferreira

Área Temática:

Versão: 5

CAAE: 03128012.0.0000.5149

Instituição Proponente: PRO REITORIA DE PESQUISA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 3.434.554

Apresentação do Projeto:

As doenças reumáticas autoimunes apresentam em sua fisiopatogenia fatores ambientais, genéticos, hormonais e infecciosos, que levam à perda da imunorregulação e à lesão de órgãos-alvo. A doença periodontal (DP) é uma infecção crônica dos tecidos de sustentação dos dentes que desencadeia, em pacientes suscetíveis, uma série de processos imunológicos dependentes da interação hospedeiro-agente infeccioso, que culminam na destruição dos tecidos afetados e na perda da inserção dentária e do osso alveolar. Trabalhos recentes descrevem mecanismos imunológicos e mecanismos de susceptibilidade genética comuns às doenças reumáticas e à doença periodontal. Por outro lado, estudos que avaliam as possíveis associações clínicas, imunológicas, genéticas e microbiológicas entre estas doenças apresentam resultados controversos. A influência da presença e gravidade da DP sobre o grau de atividade e gravidade das doenças reumáticas e vice-versa , os mecanismos inflamatórios, a ocorrência de alterações genéticas comuns, além da influência dos agentes infecciosos da DP sobre as doenças reumáticas autoimunes não são bem conhecidas e novos estudos são necessários. O presente trabalho tem como objetivo avaliar a relação entre doença periodontal e doenças reumáticas. Para isso, serão utilizados testes que mensuram atividade e cronicidade das doenças reumáticas, fluxo salivar,

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identificação e quantificação de citocinas na saliva e no sangue, avaliação de polimorfismos genéticos, estudos microbiológicos e parâmetros clínicos de doença periodontal.

Objetivo da Pesquisa:

Objetivo Primário:

1. Avaliar a influência da doença periodontal (DP) sobre a atividade e gravidade da artrite reumatóide (AR) e vice-versa.
2. Avaliar o impacto do tratamento da doença periodontal sobre a atividade da artrite reumatoide e vice-versa.

Objetivo Secundário:

1. Realizar a dosagem de citocinas pró-inflamatórias e anti-inflamatórias na saliva e no fluido crevicular e avaliar sua correlação com a presença, atividade e gravidade da DP e AR.
2. Identificar e quantificar periodontopatógenos associados à DP e à microbiota intestinal e avaliar sua correlação com a resposta imunológica, a atividade e a gravidade da DP e AR.
3. Realizar pesquisa da distribuição de polimorfismos genéticos do receptor Fcgama (alelos FcgamaRIIaR131 e FcgamaRIIB-232T) no sangue e/ou em células da mucosa bucal dos pacientes incluídos no estudo e sua relação com a DP e AR.
4. Quantificar volume e qualidade de saliva, além de avaliar possíveis associações entre DP e AR nestes parâmetros salivares.
5. Avaliação longitudinal do efeito do tratamento da DP sobre a AR e vice-versa.

Avaliação dos Riscos e Benefícios:

Riscos:

A avaliação médica será realizada através de exame clínico e aplicação de questionários e não acarretam riscos aos pacientes além dos incômodos associados ao tempo necessário à sua aplicação. Os procedimentos previstos no exame odontológico oferecem baixo risco aos pacientes, pois são minimamente invasivos e serão conduzidos por cirurgião-dentista com experiência. Durante a aferição dos indicadores periodontais, poderá ocorrer pequeno sangramento gengival. Caso aconteça, o mesmo será estancado com compressa de gaze estéril até que cesse. Os materiais utilizados estarão estéreis e todos os procedimentos serão realizados por profissional especializado respeitando as normas universais de controle de infecção.

Benefícios:

A todos os pacientes examinados será oferecida a oportunidade de tratamento odontológico nas

clínicas odontológicas de periodontia da Faculdade de Odontologia da Universidade Federal de Minas Gerais (FO-UFMG). Após o término do tratamento indicado, o nome de todos os pacientes será incluído no programa de manutenção da FO-UFMG. Os pacientes tratados serão reavaliados pelos pesquisadores para mensuração de todos os parâmetros avaliados no presente estudo. O tratamento da artrite reumatóide não sofrerá alterações devido aos achados do presente estudo, mas apenas se houver necessidade, diante do quadro clínico do paciente, conforme avaliado pela equipe médica responsável por seu acompanhamento e tratamento usuais.

Comentários e Considerações sobre a Pesquisa:

Trata-se de uma Emenda a um projeto previamente aprovado pelo Comitê de Ética.

Projeto relevante para as áreas de Reumatologia e Periodontia.

Sobre as alterações solicitadas, de acordo com os autores:

"Tipo de Alteração: Adendo para solicitar autorização na metodologia do trabalho supracitado e realização de coletas adicionais.

Justificativas: Um dos mecanismos de ligação entre as doenças periodontais e a Artrite Reumatoide (AR) envolve a disbiose no microbioma oral, a qual é parcialmente revertida após o tratamento com medicamentos modificadores do curso da doença. Apesar do significante avanço no tratamento da AR empregando-se estes medicamentos, verifica-se que cerca de 30-40% dos pacientes descontinuam o tratamento devido à ineficácia ou eventos adversos. Embora existam sugestões de mecanismos que expliquem a falha no tratamento, estas ainda não são conclusivas. Evidências recentes apontam o papel do microbioma intestinal na doença, bem como influências do microbioma oral no microbioma intestinal. Desta forma, a análise do microbioma intestinal juntamente com o microbioma oral de pacientes com AR em diferentes terapias pode apontar diferenças especificas em pacientes responsivos e não responsivos. No projeto principal a avaliação de mediadores inflamatórios estava prevista apenas utilizando a saliva, entretanto a literatura tem mostrado mais recentemente que o fluido crevicular gengival (FCG) (presente no sulco gengival que circunda o dente) reflete de forma mais específica as alterações inflamatórias dos tecidos periodontais. Desta forma, a análise do FCG poderia incorporar dados mais robustos ao projeto.

Solicitação: Com base no exposto vimos solicitar a coleta adicional de 1) fezes totais, as quais serão trazidas pelo paciente em uma das consultas de rotina médica. O paciente será instruído sobre o procedimento e receberá todo material apropriado para realizar a coleta da mesma forma que feita para exame de fezes de rotina. 2) fluido crevicular gengival, a qual será realizada durante a avaliação odontológica sob isolamento relativo com rolos de algodão e sugador. Os dentes serão

secos com gaze estéril e o FCG será coletado com fitas de papel absorvente periopaper durante 30 segundos, inseridas 1mm ou até atingir resistência nos sítios mésio-vestibular e disto-vestibular. Destaco que ambas as coletas tratam-se de procedimentos não invasivos que levam a pequenos desconfortos ou riscos para os pacientes, principalmente associados ao tempo necessário para sua realização. Os indivíduos que apresentarem periodontite serão submetidos ao tratamento periodontal gratuito. O tratamento periodontal visa ao controle da periodontite e será realizado por profissionais experientes, minimizando possíveis danos ou riscos extras provenientes deste procedimento."

No TCLE informa que todo o material proveniente dessa pesquisa será armazenado na Faculdade de Odontologia da UFMG.

Foi acrescentada no TCLE a informação: "Todo o material biológico humano coletado para estudos a serem realizados nesse projeto de Pesquisa, será depositado em Biorrepositório a ser gerenciado pela pesquisadora Dra. Tarcília Aparecida Silva, Faculdade de Odontologia da UFMG, autarquia federal, CNPJ nº 17.217.985/0001-04, conforme definido na legislação competente, atendendo, em especial, ao disposto nas Resoluções nº 441/11 e nº 466/12, ambas do CNS. Todo material será descartado após o uso na pesquisa."

Foi incluído o termo de constituição de biorrepositório, conforme orientado no parecer número 3.034.093. No entanto, foram descritas no biorrepositório apenas as amostras de saliva e fluido crevicular, não mencionando as amostras de fezes: "O Biorrepositório, constituído por amostras de saliva e fluido crevicular gengival, será sediado e armazenado na Faculdade de Odontologia da UFMG".

Considerações sobre os Termos de apresentação obrigatória:

Foram apresentados os seguintes documentos:

- Informações Básicas do Projeto;
- TCLE alterado;
- Carta-resposta às diligências da Emenda;
- Carta-resposta 2 às diligências da Emenda;
- Declaração de Manuseio Material Biológico / Biorrepositório;
- Projeto Detalhado / Brochura Investigador;
- Ficha de avaliação transversal do paciente do Ambulatório de artrite reumatóide do Serviço de reumatologia do Hospital das Clínicas – UFMG (protocoloar);
- Carta de Emenda / Adendo ao Comitê de Ética e Pesquisa da UFMG (adendocoepodontoar);
- Parecer Anterior - parecer consubstanciado do CEP da UFMG aprovado anteriormente, em 2012

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Continuação do Parecer: 3.434.554

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Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BELO HORIZONTE, 03 de Julho de 2019

Assinado por:
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(Coordenador(a))

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