UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS ODONTOLÓGICAS

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AUTOCONTROLE DE PLACA COM ESCOVA E FIO DENTAL REALIZADO POR INDIVÍDUOS TREINADOS: 6 MESES DE ACOMPANHAMENTO

Santa Maria, RS
2021

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Tese de Doutorado apresentada ao Curso de Doutorado do Programa de Pós- Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, Ênfase em Periodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor(a) em Ciências Odontológicas**

Orientador: Prof. Dr. Karla Zanini Kantorski

Santa Maria, RS
2021

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Reiniger, Ana Paula Pereira
AUTOCONTROLE DE PLACA COM ESCOVA E FIO DENTAL REALIZADO POR INDIVÍDUOS TREINADOS: 6 MESES DE ACOMPANHAMENTO / Ana Paula Pereira Reiniger.- 2021.
99 p.; 30 cm

Orientadora: Karla Zanini Kantorski
Coorientador: Carlos Heitor Cunha Moreira
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Ciências Odontológicas, RS, 2021

1. Periodontia 2. Gengivite 3. Higiene Bucal I. Kantorski, Karla Zanini II. Moreira, Carlos Heitor Cunha III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Ana Paula Pereira Reiniger

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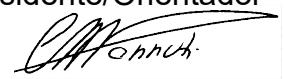

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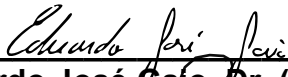

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Santa Maria, RS
2021

DEDICATÓRIA

Dedico este trabalho...

À **Deus**,
Pelo dom da vida!
Por todo amparo e proteção.
Por me guiar em todos os momentos, me trazendo paz e serenidade nos dias de angústia e dificuldades.
Por colocar em cada etapa da minha trajetória pessoas muito especiais que só me fizeram crescer e evoluir!

Aos **meus Pais**,
Regina Pereira Reiniger e Cezar Augusto Petersen Reiniger,
Obrigada pelo amor incondicional de vocês!
Vocês são meus maiores exemplos!
Com vocês aprendi a lutar pelos meus objetivos e sonhos, nunca desistir e que sempre devemos fazer o correto, o que é justo!
Amo muito vocês!

à minha avó materna,
Gleci Sittoni,
Obrigada por ser tão especial na minha vida!
Posso dizer que é minha segunda mãe.
Obrigada por todo teu amor e por tornar os momentos sempre mais doces!

Ao **meu irmão e minha cunhada**,
Rodrigo Pereira Reiniger e Luisa Lourensi,
Obrigada pelo companheirismo, por todo amor e carinho de vocês!

Ao **meu namorado**,
Gustavo Figueira Andrade,
Agradeço muito a Deus por ter colocado alguém tão especial para dividir a vida comigo!
Obrigada por tornar meus dias mais leves e cheios de amor!
Obrigada por me ajudar na "busca" da minha melhor versão, por me lembrar todos os dias dos verdadeiros valores desta vida!
Te amo muito!

AGRADECIMENTOS ESPECIAIS

À **minha orientadora**,
Karla Zanini Kantorski.
Agradeço pela oportunidade de participar de pesquisa ainda na minha graduação. Assim, pude perceber o quanto gostava e tinha interesse em fazer pós graduação.
Agradeço por todos teus ensinamentos tanto na clínica quanto em pesquisa, por compartilhar teus conhecimentos e pela dedicação à minha formação.
As suas aulas teóricas e a maneira didática que você ensina a periodontia são fascinantes, torna o que é difícil em algo fácil de compreender.
Te agradeço por estes 6 anos de convivência, por sempre ser uma orientadora tão compreensiva, humana.
Por todas as vezes que vibrou junto comigo nas minhas vitórias, mas também sempre me mostrou a realidade e o melhor caminho para contornar meus dias não tão felizes.
Esses anos de pós-graduação foram muito importantes para meu crescimento pessoal e profissional e a senhora tem um papel muito importante em tudo isso!!
Te agradeço muito!

Ao **meu Coorientador**,
Carlos Heitor Cunha Moreira.
Agradeço por toda sua contribuição na minha formação, pela paciência e por estar sempre disposto a ajudar.
É nítido o quanto o senhor ama o que faz!
Muito obrigada!

Aos **professores da banca,**
Cláudio Pannuti, Patrícia Weidlich, Eduardo Gaio, Camila Sfreddo, Patrícia Angst e Raquel Antoniazzi por aceitar nosso convite e por todas contribuições que com certeza farão muita diferença ao nosso trabalho.

Ao **Grupo da Periodontia,**
Agradeço pelo companheirismo, amizade e troca de conhecimentos.
Com vocês a pós graduação se torna mais leve.
Tenho muito orgulho de dizer que faço parte deste grupo.

Aos **professores da Periodontia,**
Raquel Antoniazzi e Fabrício Zanatta,
Agradeço por todos ensinamentos e dedicação à pós graduação e aos alunos.

Ao **Grupo do Projeto do Fio,**
Ananda Londero, Rodrigo Tavares, Gabriela Ortigara, Karen Tatsch e Ciandra Miraglia Ferreira,
Posso dizer que ganhei grandes amigos com este projeto!
Já sinto falta das nossas tardes de atendimentos, noites no Otão e das nossas jantas!
Obrigada por tornarem meus dias mais alegres!
Graças a vocês que este projeto teve êxito!

À **Camila Sfreddo,**
Estou muito feliz de ter alguém especial e que fez parte da minha trajetória neste dia tão importante na minha vida!
Obrigada por aceitar nosso convite, por toda tua ajuda e por compartilhar teus conhecimentos comigo.
Tu és um exemplo de profissional para todos nós!

À **minha super amiga**,
Juliana Maier,
Não me canso de agradecer a Deus por ter te colocado no meu caminho!
Meu mestrado foi maravilhoso e com certeza você foi um dos grandes motivos.
Obrigada pela tua amizade, pela troca de conhecimentos e por tudo!
Tu é uma das pessoas que me faz confirmar o que sempre acreditei: "Trabalhar juntos e torcer pelo outro é sempre o melhor caminho!"
Tenho muito orgulho de ti amiga!

À **minha amiga querida,**
Tatiana Militz,
Agradeço muito pela tua amizade, companheirismo e por compartilhar as aulas do doutorado comigo.
Sinto muita saudade da nossa convivência.
Tu és uma pessoa de um coração gigante, muito especial!

À Fernanda Tomazoni,
Sempre digo que Deus coloca pessoas muito especiais no meu caminho, você com certeza é uma delas!
Obrigada pela tua amizade, por todos os momentos que passamos juntas no Departamento de Morfologia.
O que para mim parecia algo muito difícil, se tornou muito mais tranquilo com a tua ajuda!
Muito obrigada!

À Jessica Dalcin,
Obrigada pela tua disponibilidade e por sempre estar disposta a nos "salvar".
Obrigada por ter sido nossa paciente do projeto, por me ajudar a configurar imagens para artigos, por estar sempre pronta para ajudar!
Tu és muito especial e importante para o Programa e para os alunos da Pós-Graduação!

Aos meus avós paternos,
Maria Amélia Petersen Reiniger e Carlos Armando Petersen Reiniger,
Agradeço todo o amor de vocês!

A minha dinda,
Ana Lúcia Garrastazu Pereira,
Obrigada por todo teu carinho, por ser uma dinda tão especial e presente na minha vida!

A minha família,
Tios, tias e primos,
Agradeço por serem tão presentes e especiais na minha vida!

A família do meu namorado,
Susana, Paulo, Marcela, Pablo, Miguel e Laurinha,
Obrigada pelo carinho de vocês e por me fazer sentir parte da família!

A minha filha pet, Fiona
Sou muito grata por ter ela na minha vida!
Torna meus dias sempre cheios de amor!

AGRADECIMENTOS

À **Universidade Federal de Santa Maria**,

Sou muito grata a esta Universidade onde me formei em odontologia, pude cursar dois cursos de pós graduação (mestrado e doutorado) e ainda tive o privilégio de ser professora substituta.
Tenho muito orgulho de ser "filha" da UFSM!

A **Pós Graduação PPGCO**,
Pela oportunidade de fazer mestrado e doutorado neste programa.
Pelo ensino de alta qualidade.
Tenho certeza que tive uma excelente formação.

Aos professores e ao curso de Odontologia,
Obrigada por toda dedicação e ensinamentos aos alunos!

Àos **professores da Pós Graduação em Ciências Odontológicas,**
Muito obrigada por todo conhecimento e experiências compartilhadas!
Vocês são muito importantes para nossa formação profissional e pessoal!

A **Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES),**
Pelo apoio proporcionado para realização deste trabalho e pela bolsa de estudos durante todo mestrado e 3 anos de doutorado.

Aos **Pacientes dos projetos,**
Agradeço pela disponibilidade de participar dos nossos estudos.
Vocês foram fundamentais para que estes trabalhos se tornassem reais!

RESUMO

AUTOCONTROLE DE PLACA COM ESCOVA E FIO DENTAL REALIZADO POR INDIVÍDUOS TREINADOS: 6 MESES DE ACOMPANHAMENTO

AUTOR: Ana Paula Pereira Reiniger
ORIENTADORA: Karla Zanini Kantorski

A presente tese teve como objetivo responder, por meio de dois artigos científicos, questões referentes à prevenção e tratamento da inflamação gengival através do autocontrole de placa em dois perfis de sujeitos. O primeiro artigo teve como objetivo avaliar se indivíduos sem perda de inserção prévia que passaram por intensivo treinamento de higiene bucal (HB) (8 sessões semanais) (a) estariam aptos a manter os novos hábitos de HB durante 180 dias sem intervenção profissional e (b) se o fio dental permaneceria resultando em benefícios adicionais à escovação durante este período. Setenta e cinco indivíduos com mínimo de 15% de sítios proximais com sangramento gengival foram randomizados para receber o treinamento com escova multicerdas (E) ou escova multicerdas+fio dental (E/F). A seguir, os indivíduos foram acompanhados durante 180 dias sem intervenção profissional. Médias de escore 2 do Índice Gengival (IG) (sangramento gengival) e de IG proximal foram os desfechos primários. Comparação entre os grupos foi avaliada por Modelos lineares mistos ($p < 0,05$). Quarenta e oito sujeitos completaram o acompanhamento. Após redução de inflamação gengival associada ao período de treinamento, nenhuma alteração na condição gengival foi observada dentro dos grupos durante 180 dias, mostrando que os indivíduos mantiveram os hábitos de higiene bucal aprendidos. Em 180 dias, o uso adjunto do fio dental (12,8±2,5), mostrou benefícios adicionais à escova (19,8±2,2) considerando diferenças estatisticamente significantes no sangramento gengival proximal. Quando pacientes com a papilas intactas são treinados para usar adequadamente o fio dental, o fio associado com a escova promove maior redução da inflamação gengival proximal quando comparado a escova. O segundo artigo avaliou, em indivíduos com histórico de periodontite inclusos em manutenção periodontal com máximo de 7,5% de sítios com escore 2 do IG, a correlação entre acúmulo de placa e a condição gengival. Quarenta e dois indivíduos foram randomizados para realizar HB em intervalos diários (12h e 24h) ou estendidos (48h). Índice de Placa (IP), IG e Sangramento a Sondagem (SS) foram mensurados no baseline, 30 e 90 dias. Estatísticas descritivas e correlação de Spearman entre acúmulo de placa e condição gengival foram apresentadas. Ambos os grupos, G12/24h e G48h, mostraram aumento significativo nos escores de placa ao longo do estudo, mas somente indivíduos com intervalos de HB estendidos apresentaram aumento da inflamação gengival (SS e escore 2 do IG). Aos 90 dias, G48h apresentou escores estatisticamente maiores de inflamação gengival do que o G12/24h. Enquanto as correlações entre IP/IG não foram afetadas pelos diferentes intervalos de HB, as correlações entre IP/SS e IP/escore 2 do IG permaneceram imutáveis no G12/24h e aumentaram no G48h. Isso significa que indivíduos realizando HB em intervalos estendidos permitem suficiente desenvolvimento de placa para resultar em inflamação gengival; enquanto a HB diária, embora possa resultar em aumento dos escores de placa, não permite o desenvolvimento da mesma a uma condição que induza resposta inflamatória gengival.

Palavras-chave: Doenças Gengivais. Fio dental. Gengivite. Higiene bucal. Placa bacteriana. Periodontite

ABSTRACT

SELF-CONTROL OF PLAQUE WITH TOOTHBRUSH AND DENTAL FLOSS CARRIED OUT BY TRAINED INDIVIDUALS: 6 MONTHS OF FOLLOW-UP

AUTHOR(A): Ana Paula Pereira Reiniger
ADVISOR: Karla Zanini Kantorski

This thesis aimed to answer, through two scientific articles, questions regarding the prevention and treatment of gingival inflammation through self-control of plaque in two patient profiles. The first article aimed to assess whether individuals without prior attachment loss who underwent intensive oral hygiene (OH) training (8 weekly sessions) (a) would be able to maintain the new OH habits for 180 days without professional intervention and (b) whether dental floss would remain resulting in additional benefits to brushing during this period. Seventy-five subjects with a minimum of 15% proximal sites with gingival bleeding were randomized to receive toothbrush (TB) or toothbrush+dental floss (TB+) training. Then, the individuals were followed for 180 days without professional intervention. Proximal Gingival Index (GI) score 2 (gingival bleeding) was the primary outcome. Comparison between groups was assessed by Mixed Linear Models ($p < 0.05$). Forty-eight subjects completed follow-up. After reduction of gingival inflammation associated with the training period, no change in gingival condition was observed within the groups for 180 days, showing that the subjects maintained the learned oral hygiene habits. At 180 days, the adjunct use of dental floss (12.8±2.5) showed additional benefits to the toothbrush (19.8±2.2) considering statistically significant differences in proximal gingival bleeding. When patients with intact papillae are trained to properly dental floss, the dental floss associated with the toothbrush promotes a greater reduction in proximal gingival inflammation when compared to the toothbrush. The second article evaluated, in individuals with a history of periodontitis included in periodontal maintenance with a maximum of 7.5% of sites with a GI score of 2, the correlation between plaque accumulation and gingival condition. Forty-two individuals were randomized to perform OH at daily (12h and 24h) or extended (48h) intervals. Plaque Index (PI), GI and Bleeding on Probing (BoP) were measured at baseline, 30 and 90 days. Descriptive statistics and Spearman correlation between plaque accumulation and gingival condition were presented. Both groups, G12/24h and G48h, showed a significant increase in plaque scores throughout the study, but only individuals with extended OH intervals had increased gingival inflammation (BoP and IG score 2). At 90 days, G48h had statistically higher gingival inflammation scores than G12/24h. While the correlations between PI/GI were not affected by the different OH intervals, the correlations between PI/BoP and PI/GI score 2 remained unchanged in G12/24h and increased in G48h. This means that individuals taking OH at extended intervals allow enough plaque development to result in gingival inflammation; while daily OH, although it may result in increased plaque scores, does not allow the development of the same to a condition that induces gingival inflammatory response.

Key words: Dental floss. Dental plaque. Gingivitis. Gingival Disease. Periodontitis. Oral hygiene.

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SUMÁRIO

1 INTRODUÇÃO E JUSTIFICATIVA

A gengivite induzida pela placa é uma resposta inflamatória dos tecidos gengivais resultante do acúmulo de biofilme bacteriano localizado na margem gengival (LÖE; THEILADE; JEPSEN, 1965; MURAKAMI et al., 2018). A etiologia bacteriana da gengivite foi estabelecida em modelo de gengivite experimental em humanos por Löe e colaboradores em 1965. Indivíduos com saúde gengival deixaram de realizar seus procedimentos de higiene bucal (HB) permitindo o livre acúmulo de placa sobre a gengiva. Em um período que variou de 10 a 21 dias, todos os participantes desenvolveram gengivite; e após 7 a 10 dias do retorno de adequados procedimentos de HB, a saúde gengival foi reestabelecida. Na década de 70, Lindhe e colaboradores introduziram o conceito de que a gengivite, se não tratada, pode evoluir para a periodontite, onde destruição permanente dos tecidos de suporte dentários é observada (LINDHE; HAMP; LOE, 1975). Evidências longitudinais subsequentes elucidaram a história natural das doenças periodontais no homem, indicando que entre populações sem essenciais cuidados de HB e sem atenção odontológica, nas quais substancial acúmulo de placa e cálculo são evidentes, a maioria dos indivíduos desenvolve periodontite leve a moderada, enquanto uma pequena subpopulação apresenta perdas de inserções mais severas (BAELUM; FEJERSKOV; KARRING, 1986; LÖE et al., 1986). Tomados em conjunto, esses conceitos indicam que o acúmulo de placa sobre os dentes é um pré-requisito para iniciação e desenvolvimento da gengivite e progressão desta para periodontite. Assim, gengivite apresenta particular significância desde que é considerada a precursora da periodontite. Corroborando esse conceito, dados longitudinais têm demonstrado que melhoras no controle de placa reduziram não somente gengivite, mas também periodontite (HUGOSON; SJODIN; NORDERYD, 2008, RAMSEIER et al., 2017) e que dentes associados com unidades gengivais inflamadas apresentam maior risco para serem perdidos quando comparados aqueles associados com gengivas saudáveis (SCHÄTZLE et al., 2003).

Mais recentemente, evidências sugerem que há diferentes perfis individuais de suscetibilidade ao acúmulo da placa. Trombelli et al., 2004 demonstraram que exposição à similares quantidades de placa produziram diferentes expressões clínicas de gengivite, classificando subpopulações como “high responders” e “low responders”. Isso implica que pode não haver uma correlação tão direta entre acúmulo de placa de

inflamação gengival. A compreensão da correlação entre acúmulo de placa e inflamação gengival de acordo com diferentes fatores é fundamental porque na rotina clínica odontológica os escores de placa são utilizados para estabelecer a necessidade de modificações de comportamento do paciente e indicar intervenções terapêuticas, sendo isso de particular importância para pacientes periodontais durante a manutenção.

Os principais sinais e sintomas da gengivite são aqueles classicamente associados com inflamação. Pacientes podem relatar sangramento durante a escovação, sangue na saliva, vermelhidão, edema e halitose (MURAKAMI et al., 2018). Alterações decorrentes da gengivite são restritas à gengiva e são reversíveis com a remoção do fator etiológico sem prejuízo ao periodonto de suporte (MARIOTTI, 1999). Dados epidemiológicos têm demonstrado alta prevalência de gengivite nas populações em países desenvolvidos e em desenvolvimento (LI et al., 2010; MINISTÉRIO DA SAÚDE, 2011, TROMBELLI et al., 2018), sendo o tipo mais frequente de doença periodontal (PAGE; BAAB, 1985).

O principal mecanismo para prevenir a gengivite é a auto remoção de placa realizada de forma periódica em casa por meio dos procedimentos de HB. Para a HB ser efetiva, dois fatores são necessários: (i) desorganizar mecanicamente a placa de todas as superfícies dentárias e (ii) e fazer esse procedimento com uma frequência adequada (JEPSEN, 1998).

Quanto a periodicidade da HB, evidências têm indicado que quando um adequado autocontrole mecânico da placa é realizado, a frequência de 1 vez ao dia é suficiente para manter saúde gengival em indivíduos sem (PINTO et al. 2013, DE FREITAS et al., 2016) e com (MAIER et al., 2020) histórico de periodontite. O *XI European Workshop of Periodontology* recomenda escovação diária duas vezes ao dia com dentifrício fluoretado (CHAPPLE et al., 2015); e a American Dental Association (ADA) (2016) recomenda escovação diária duas vezes ao dia e uso de dispositivo interdental uma vez ao dia.

Quanto a remoção efetiva da placa, a escova multicerdas é o instrumento mais utilizado (VAN DER WEIJDEN; SLOT, 2015). Embora a escova multicerdas seja o dispositivo indicado para faces livres, faces interproximais exigem dispositivos específicos devido a presença de fibras apicais ao ponto de contato entre dentes adjacentes, que dificultam o acesso das escovas possibilitando o estabelecimento e maturação da placa bacteriana (SALZER et al., 2015). Assim, a remoção de placa

interdental com dispositivos específicos é reconhecida como parte essencial na manutenção de saúde gengival (AMERICAN DENTAL ASSOCIATION, 2016). O fio dental tem sido preconizado para pacientes sem perda de inserção proximal, cujas papilas preenchem todo espaço interdental (SÄLZER et al., 2015).

Apesar do conhecimento público sobre a necessidade do fio dental para adequada HB, há alta prevalência de gengivite proximal (LI et al., 2010). Isso significa que as pessoas não utilizam o fio dental ou utilizam de maneira inadequada. Em geral, o uso diário do fio é pouco aceito na rotina dos pacientes (ASADOORIAN; LOCKER, 2006). Estudos mostram que a baixa cooperação ocorre devido à falta de motivação dos indivíduos (ASADOORIAN; LOCKER, 2006) e as dificuldades técnicas do uso do dispositivo interdental (ETHAN et al., 2019). Sambunjak et al., 2011 afirmam que muitos pacientes usam o fio dental de forma incorreta, sendo o dispositivo utilizado rapidamente entre os pontos de contato, não desorganizando suficientemente a placa bacteriana a fim de manter saúde nas superfícies proximais.

Alguns ensaios clínicos randomizados (SCHIFF et al., 2006; GRAZIANI et al., 2018) falharam em demonstrar benefício adicional do uso adjunto do fio dental à escovação na redução da inflamação gengival, enquanto outros (SHARMA et al. 2002; BAUROTH et al., 2003; ROSEMA et al., 2008) demonstraram que fio dental mais escova multicerdas promoveram maior redução da inflamação gengival quando comparados ao uso exclusivo da escova. Recentes revisões sistemáticas (SÄLZER et al., 2015; WORTHINGTON et al., 2019) sugerem que escovação associada com fio dental promove maior redução da gengivite quando comparada a escovação sozinha. Entretanto, embora os resultados tenham significância estatística, o efeito observado parece ser clinicamente não importante. Worthington et al., (2019) destacaram os baixos escores de inflamação gengival no baseline dos estudos incluídos; e ainda apontaram que a certeza da evidência de que o fio adjunto à escova reduz mais gengivite quando comparado somente a escova é baixa. Apenas um estudo avaliou o uso adjunto do fio em um período de acompanhamento maior do que 6 meses (ROSEMA et al., 2008). Entretanto, este estudo utiliza exames clínicos parciais e instruções de higiene esparsas. Os exames parciais podem enviesar a real situação de saúde gengival e poucas orientações podem ser insuficientes para treinar e motivar os pacientes a utilizar de forma adequada o dispositivo interdental. Recente meta-análise em rede (KOTSAKIS et al., 2018) afirma que o uso do fio dental sem adequada instrução profissional não reduz inflamação gengival. Por outro lado, quando o fio

dental é utilizado com a técnica adequada, a incidência de cárie por exemplo, foi reduzida em 40% em escolares (HUJOEL et al., 2006). Portanto, faz-se necessário treinamento da técnica de utilização do fio dental para que esse possa trazer algum benefício adicional à escovação.

Neste contexto de informações, a presente tese tem como objetivo responder questões sobre autocontrole da placa bacteriana para prevenção e tratamento de inflamação gengival em dois perfis de pacientes:

a) O primeiro artigo é uma análise de acompanhamento de um ensaio clínico randomizado (LONDERO et al., 2021), em que os indivíduos da amostra deveriam apresentar pelo menos 15% de gengivite proximal e papilas intactas. Os indivíduos foram randomizados em dois grupos de treinamento de HB (1 sessão por semana durante 8 semanas): escova multicerdas ou escova multicerdas suplementada com fio dental. Neste estudo prévio (LONDERO et al., 2021), nós demonstramos que a gengivite proximal pode ser significativamente reduzida pela escova e pela escova suplementada com fio, mas que o efeito é significativamente maior quando o fio dental é utilizado. Nosso objetivo no primeiro artigo da tese foi responder duas questões: (i) os indivíduos conseguiriam manter os métodos de HB aprendidos durante o período de treinamento durante 180 dias sem nenhuma intervenção profissional? (ii) o uso adjunto do fio dental continuaria proporcionando benefícios adicionais a escova como observado durante o período de treinamento?
b) O segundo artigo da tese é uma análise secundária de um ensaio clínico randomizado (MAIER et al. 2020) que teve como objetivo avaliar o efeito de diferentes intervalos de HB na saúde gengival em pacientes periodontais tratados e submetidos à manutenção periodontal. Nosso objetivo aqui foi avaliar a correlação entre acúmulo de placa bacteriana e inflamação gengival nesse perfil de pacientes os quais realizavam os procedimentos de HB em intervalos diários (12/24h) e estendidos (48h).

2 REVISÃO DE LITERATURA

2.1 INFLAMAÇÃO GENGIVAL INDUZIDA POR PLACA BACTERIANA

Gengivite é uma inflamação gengival induzida por placa bacteriana, resulta da interação entre o biofilme bacteriano e a resposta imunoinflamatória do hospedeiro (CHAPPLE et al., 2018). Esta condição permanece confinada a gengiva, não se estendendo além da junção mucogengival e ao periodonto de suporte (cemento, ligamento periodontal e osso alveolar) (CHAPPLE et al., 2018). Apresenta caráter reversível (TATAKIS et al., 2004), através da redução nos níveis de placa bacteriana dentária (CHAPPLE et al., 2018). É a forma mais comum de doença periodontal e altamente prevalente em diferentes populações (GJERMO et al., 2002; SUSIN et al., 2004, TONETTI et al., 2017).

A etiologia da gengivite foi estabelecida em um estudo clássico de gengivite experimental em 1965 por Löe et al. Este estudo demonstrou que o acúmulo de microrganismos e sua persistência na margem gengival por um período mínimo de 10 a 21 dias causa inflamação gengival. A saúde dos tecidos periodontais é reestabelecida após o retorno de medidas de higiene bucal, e consequente desorganização da placa supragengival, entre 7 a 10 dias (LÖE; THEILADE; JENSEN, 1965).

Edema, eritema, sangramento, sensibilidade e aumento do volume gengival são sinais e sintomas clínicos comuns de inflamação gengival (TONETTI et al., 2015; SUZUKI 1988). Existe uma variabilidade entre os indivíduos quanto aos sinais e sintomas clínicos (TROMBELLI et al., 2004), assim como entre os sítios da mesma dentição (MURAKAMI et al., 2018). As alterações histopatológicas incluem a proliferação de células basais no epitélio juncional, vascularização dos vasos sanguíneos adjacentes ao epitélio juncional, destruição progressiva das fibras colágenas, alterações citopatológicas dos fibroblastos residentes e presença de intenso infiltrado inflamatório (PAGE; SCHROEDER, 1976).

A gravidade da gengivite induzida por placa pode ser influenciada por diversos fatores (MARIOTTI, 1999). Alguns têm sido relacionados ao aumento e gravidade da inflamação, como: diabetes mellitus (CARNEIRO et al., 2015), alterações hormonais presentes na puberdade (CHAITRA et al., 2012), período menstrual (BECERIK et al., 2010) e gravidez (CARRILLO-DE-ALBORNOZ et al., 2012). O fumo tem sido

associado como um importante fator de risco para doenças periodontais (MURAKAMI et al., 2018) e pode mascarar a inflamação gengival porque reduz os sinais inflamatórios (PERUZZO et al., 2016). Alguns medicamentos (ciclosporina, fenitoina e nifedipina, verapamil) têm sido associados com aumento de volume gengival periodontais (MURAKAMI et al., 2018).

Inflamação gengival é um pré-requisito necessário para o desenvolvimento subsequente de periodontite, progressiva perda de inserção ao redor dos dentes (TROMBELLI et al., 2018). Entretanto, nem todos os casos de gengivite irão progredir para periodontite (BROWN; LÖE, 1993; PRAYITNO; ADDY; WADE, 1993). Isto ocorre porque a placa não é suficiente para o desenvolvimento da periodontite, sendo necessária também a presença de um hospedeiro suscetível (PAGE; SCHROEDER, 1981, TROMBELLI et al., 2004). O tratamento da gengivite é uma estratégia preventiva primária para periodontite e uma estratégia secundária para periodontite recorrente (MARIOTTI 1999; LOE; THEILADE; JEPSEN, 1965).

Segundo a classificação do Workshop Mundial de 2017, a gengivite pode ser classificada como: gengivite em um periodonto intacto, gengivite em um periodonto reduzido em um paciente sem periodontite ou inflamação gengival em um periodonto reduzido em um paciente com histórico de periodontite e tratado com sucesso (CHAPPLE et al., 2018).

2.2 GENGIVITE EM UM PERIODONTO INTACTO E REDUZIDO SEM HISTÓRICO DE PERIODONTITE

Para um paciente ser classificado como caso de gengivite em um periodonto intacto, ele não pode ter perda de inserção clínica, nem perda óssea radiográfica, e apresenta:

- Localizada: Sangramento a sondagem positivo em 10 a 30% dos sítios;
- Generalizada: Sangramento a sondagem positivo em mais de 30% dos sítios;

Já o paciente com periodonto reduzido sem histórico de periodontite, além de apresentar sangramento à sondagem positivo em ≥10% dos sítios, apresenta perda de inserção e perda óssea radiográfica devido à recessão gengival ou alongamento de coroa por exemplo. Este paciente também pode ser classificado em localizada (10-30%) ou generalizada (>30%) (TROMBELLI et al., 2018).

2.3 INFLAMAÇÃO GENGIVAL EM UM PERIODONTO REDUZIDO COM HISTÓRICO DE PERIODONTITE

A periodontite é uma doença inflamatória crônica multifatorial com biofilme disbiótico e caracterizada por destruição progressiva dos tecidos de suporte dentários (PAPAPANOU et al., 2018). Segundo a classificação das doenças periodontais da Academia Americana de Periodontia e Federação Europeia de Periodontia realizada em 2017, o paciente com histórico de periodontite estável que apresenta inflamação gengival marginal permanece sendo considerado um caso de periodontite (CHAPPLE et al., 2018).

A redução da inflamação e estabelecimento de saúde gengival em um periodonto reduzido pode ser alcançada em 2 níveis, denominados estabilidade e remissão/controle. O paciente considerado como estável é definido como um estado em que a periodontite foi tratada com sucesso através do controle de fatores sistêmicos e locais. Este paciente tem baixos (mínimos) índices de SS positivos, redução na profundidade de sondagem e nível de inserção clínica e não apresenta progressão da doença. Além disso, controle de fatores modificadores como a redução de fumo e adequado controle de diabetes são alcançados. Já o paciente considerado como remissão/controle é definido como um período em que o curso da doença durante o tratamento resultou na redução de inflamação e melhorias na PS e NIC, mas não alcança um ótimo controle de fatores locais e sistêmicos (LANG; BARTOLD, 2018)

2.4 PREVENÇÃO E TRATAMENTO

A desorganização efetiva da placa bacteriana através restabelecimento de hábitos adequados de higiene bucal é o principal mecanismo de prevenção e tratamento da gengivite (SAMBUNJAK et al., 2011). O autocontrole mecânico de placa, utilizando escovas multicerdas e dispositivos interdentais, é fundamental para a redução da presença de inflamação no periodonto e redução do risco de perda de inserção e perda dentária futura (HUGOSON; SJODIN; NORDERYD, 2008). Porém, a capacidade do indivíduo em desorganizar a placa bacteriana de todas as superfícies dentárias e a frequência que este autocontrole mecânico de placa é realizado são

fatores fundamentais para a efetividade deste procedimento (JEPSEN, 1998). American Dental Association (ADA) (2016) recomenda escovação dentária duas vezes ao dia e dispositivo interdental uma vez ao dia para prevenir cárie e gengivite, embora exista evidência limitada dessa recomendação em relação à manutenção da saúde gengival (CHAPPLE et al., 2015).

2.4.1 – Escova Multicerdas

O uso de escovas multicerdas e dentifrício fluoretado para realização da higiene bucal pelo paciente é praticamente universal. Entretanto, apesar do uso generalizado, a maioria da população não realiza o autocontrole mecânico de placa de maneira suficiente para evitar o acúmulo de placa bacteriana (MORRIS et al., 2001; CLAYDON 2008). Segundo Westfeld 1996, estes dados podem ser explicados devido a população não compreender o processo da doença ou até mesmo não saber realizar uma técnica adequada para desorganização do biofilme bacteriano.

Van der Weijden e Hioe 2005 observaram em uma revisão sistemática (estudos de seis meses de acompanhamento) um pequeno efeito na redução de gengivite após uma única orientação de higiene com escovas multicerdas. Bosma 2011 afirma que mesmo após orientação de higiene e profilaxia oral profissional, poucos pacientes conseguem manter os tecidos gengivais compatíveis com saúde. Hábitos de higiene bucal estão fortemente associados ao comportamento do indivíduo em relação a sua saúde geral (BOSMA, 2011). Portanto, é necessário estimular e conscientizar o indivíduo sobre sua responsabilidade em adequar seus hábitos de higiene e manter saúde gengival (WESTFELD, 1996; HUGOSON et al., 2007).

Alguns autores vêm buscando a melhor frequência de autocontrole mecânico de placa compatível com saúde em pacientes com adequado controle do biofilme. Lang et al., 1973 encontraram compatibilidade com saúde gengival em uma frequência de higiene bucal a cada 48 horas. Kelner et al., 1974 determinaram que a frequência de autocontrole mecânico de placa a cada 24 horas é compatível com saúde gengival e os pacientes que realizaram frequência a cada 72 horas desenvolveram sinais inflamatórios de gengivite. Entretanto, existem limitações metodológicas nestes estudos que tornam difícil a inferência destes achados para a população geral. Os pacientes tinham conhecimento da etiologia e patogênese da

doença periodontal (estudantes de odontologia), remoção de placa foi supervisionada por higienistas bucais e realizada com o uso de evidenciadores. Portanto, a cada escovação ocorreu uma remoção completa e meticulosa de placa, o que geralmente não acontece na maioria da população.

Estudos mais recentes como Pinto et al., 2013 e De Freitas et al., 2016 realizaram um ensaio clínico randomizado com estudantes universitários não pertencentes a cursos da área da saúde, sem histórico de periodontite e que apresentavam adequado controle de placa bacteriana. O status gengival foi avaliado entre os indivíduos que realizaram higiene bucal a cada 12, 24, 48 e 72 horas durante 30 dias. Os participantes dos grupos 12h e 24h mantiveram os níveis de saúde gengival. O intervalo de 48h e 72h mostrou-se não ser compatível com manutenção de saúde gengival.

Em 2020, Maier et al., acompanharam indivíduos com histórico de doença periodontal e em manutenção periodontal em três diferentes intervalos de autocontrole mecânico de placa: 12h, 24h e 48h durante 90 dias. Os resultados foram similares aos estudos realizados em pacientes sem histórico de periodontite. Os grupos que realizaram a frequência de escovação a cada 12h e 24h apresentaram condições gengivais compatíveis com saúde e o mesmo não acontece no grupo 48h.

2.4.2 – Fio Dental

O conceito de limpeza interdental com material filamentoso foi introduzido pela primeira vez por Levi Spear Parmly como uma medida para prevenir doenças dentárias associado com dentifrício e escova dentária (PARMLY, 1819). O fio dental de seda não encerado foi produzido pela primeira vez em 1882 pela Codman & Shurtleff, mas foi Johnson & Johnson quem tornou o fio de seda amplamente disponível a partir de 1887 (WORTHINGTON et al., 2019).

O fio dental tem sido indicado para remoção de placa interproximal (ASADOORIAN; LOCKER, 2006) e acredita-se que seu uso frequente reduz o risco de doenças periodontais e cáries interproximais (HUJOEL et al., 2006). Entretanto, O 11º Workshop da Federação Europeia de Periodontia relata que existem evidências fracas/inconsistentes para a eficácia do uso de dispositivos interdentais como adjuvantes a escovação dentária (CHAPPLE et al., 2015). Apesar de amplamente defendido, a maioria dos estudos disponíveis falha em demonstrar que o uso do fio

dental é eficaz na remoção da placa bacteriana e na redução da inflamação gengival (CHAPPLE et al., 2015). Uma revisão sistemática network também afirma que o uso do fio dental não supervisionado não produz reduções importantes na inflamação gengival (KOTSAKIS et al., 2018). Os autores desta revisão sistemática acreditam que o desafio de executar um hábito de higiene bucal com uma técnica exigente e difícil como o uso do fio dental poderia explicar estes resultados (KOTSAKIS et al., 2018).

Muitos estudos têm sido realizados com o intuito de avaliar a eficácia de dispositivos interdentais adjuntos à escovação dentária na remoção de placa interproximal e, consequentemente, inflamação gengival. Vogel et al., 1975 avaliaram vinte e quatro estudantes de odontologia a fim de comparar a eficácia de dispositivos de higiene oral para manutenção da saúde gengival interproximal. No período pré-baseline, os pacientes receberam instrução de higiene com fio dental, palitos de borracha e escova multicerdas a cada três dias e foram orientados a utilizar estes dispositivos uma vez por dia. No baseline, os estudantes apresentaram saúde gengival e foram randomizados em 4 grupos: I- apenas escova, II- escova e fio dental, III- escova e palito de borracha, IV- escova, fio dental e palito de borracha. Nos dias 0, 9, 15 e 33, exames clínicos e reforços de orientação de higiene foram realizados em todos os grupos. Ao final do período experimental (dia 33), os grupos não apresentaram diferença em todos os parâmetros avaliados, inclusive índice gengival interproximal. Os autores acreditam que o fato dos pacientes serem estudantes de odontologia, todos eram muito motivados e estavam familiarizados com as técnicas de higiene bucal, por isso conseguiram remover considerável acúmulo de placa interproximal dos dentes.

Em 1991, Kiger et al., avaliaram 30 pacientes adultos com histórico de periodontite tratados e em manutenção periodontal. Este ensaio clínico cross-over teve como objetivo avaliar a eficácia do fio dental e escova interdental na remoção de placa interproximal. No baseline, os indivíduos receberam profilaxia, orientação de higiene e foram randomizados em 3 grupos: escova multicerdas sozinha, escova multicerdas e fio dental, escova multicerdas e escova interdental. Após um mês do início do estudo, os pacientes retornavam para exames clínicos, profilaxia e orientação de higiene para outro regime de tratamento. O estudo continuou desta maneira por 3 meses, permitindo que os pacientes higienizassem seus dentes com cada um dos tratamentos pelo período de 1 mês. Espaços interproximais foram selecionados em cada paciente e considerados como sítios teste. Estes sítios deviam apresentar

espaço suficiente para entrar escova interdental. Os exames clínicos foram realizados apenas nos sítios teste. Os resultados indicam que escova interdental associada a escova multicerdas é mais efetiva do que apenas escova multicerdas ou escova multicerdas associada ao fio dental para remoção de placa em superfícies dentárias proximais de indivíduos tratados periodontalmente. Entretanto, estes pacientes tinham histórico de doença periodontal, espaço entre os dentes e apenas sítios testes foram avaliados.

Bauroth et al., 2003 realizaram um ensaio clínico randomizado de 6 meses de acompanhamento com intuito de comparar a eficácia do uso do fio dental versus enxaguatório bucal de óleos essenciais no controle de gengivite interproximal. Trezentos e vinte e seis sujeitos com gengivite leve a moderada foram randomizados em 3 grupos de acompanhamento: (*BEO group* – escova e enxaguatório bucal de óleos essenciais; *BF group*- escova e fio dental; *B group* – escova e enxaguatório bucal controle). No exame de baseline, fatores retentivos de placa, profilaxia dentária e instrução de higiene bucal foram realizados. No exame de 6 meses, de acordo com o Índice Gengival Modificado interproximal (LOBENE et al., 1986), o grupo *BEO* reduziu 11,1% e o grupo *BF* 4,3% de inflamação gengival em relação ao grupo *B*. O Índice de Placa modificado por Quigley-Hein (QHI) (TURESKY et al., 1970) em sítios proximais reduziu 20,0% no grupo *BEO* e 3,4% no grupo *BF* em relação ao grupo controle. Os autores concluem que o enxaguatório bucal de óleos essências obteve resultados tão bons quanto o uso do fio dental para gengivite proximal.

Zimmer et al., 2006 compararam a eficácia do fio dental e dois enxaguatórios antimicrobianos para redução de placa e gengivite interproximal durante 8 semanas. Cento e sessenta e cinco indivíduos sistemicamente saudáveis e com gengivite foram randomizados em 4 grupos: I- Escova e enxaguatório com clorexidina (0,06 clorexidina e 0,025 fluoreto de sódio); II- Escova e enxaguatório sem clorexidina (0,1 cloreto de cetilpiridínio e 0,025 fluoreto de sódio); III- Escova e fio dental; IV- escova. Os indivíduos receberam orientação de higiene bucal de acordo com o seu grupo apenas no baseline. O exame final do período de acompanhamento mostra que houve maior redução de placa nos grupos que utilizaram enxaguatório (I e II). Entretanto, não houve diferença estatisticamente significante entre os grupos quando o Índice de Sangramento Papilar (PBI) foi avaliado. Os autores sugerem que o uso de enxaguatório bucal associado a escovação diária é mais eficaz na remoção de placa interproximal do que o uso do fio dental associado a escova.

Schiff 2006 avaliaram 3 regimes de higiene oral para controle de placa e gengivite durante 6 meses. Os indivíduos precisavam apresentar média de IG = 1,0 para ser elegível para o estudo. A randomização foi realizada em três grupos: 1) Escovação duas vezes por dia com dentifrício contendo 0,3% de triclosan, 2,0% PVM/MA copolímero e 0,243% de fluoreto de sódio (Colgate® Total® Toothpast, USA) acompanhado de fio dental uma vez ao dia; 2) Escovação duas vezes por dia com dentifrício contendo 0,3% de triclosan, 2,0% PVM/MA copolímero e 0,243% de fluoreto de sódio (Colgate® Total® Toothpast, USA) sem usar o fio dental; 3) Escovação duas vezes por dia com dentifrício contendo 0,243% de fluoreto de sódio (Crest® Fluoride Toothpaste, USA) acompanhado de fio dental uma vez ao dia. Após a randomização e exames do baseline, os pacientes receberam profilaxia oral completa. Após 3 e 6 meses, exames de placa e inflamação gengival foram realizados novamente. Cento e quatorze pacientes completaram o protocolo do estudo durante os 6 meses. Após 3 e 6 meses, os grupos que utilizaram o dentifrício com triclosan (Colgate® Total® Toothpast, USA) independente de usar ou não o fio dental (1 e 2) apresentaram menores índices gengivais do que o grupo que utilizou o dentifrício fluoretado (Crest® Fluoride Toothpaste, USA) (3) e estes resultados foram estatisticamente significantes. Os grupos 1 e 2 não apresentaram diferença estatistica nos dois exames. Entretanto, o grupo 1 que utilizou fio dental demonstrou escores de IG levemente menores em relação ao grupo 2. Estes indivíduos receberam apenas orientação sobre a quantidade de vezes para utilizar o fio dental e dentifrício de acordo com cada grupo. Instrução de higiene, especialmente em relação ao fio dental, pode ter sido um fator importante para não haver diferença entre os grupos.

Hague e Carr 2007 compararam a eficácia de um fio dental automático comparado ao fio dental manual. Este ensaio clínico crossover avaliou 102 pacientes saudáveis periodontalmente durante 10 semanas em três diferentes grupos de tratamentos: *C:* controle (apenas escova manual), *M*: Escova manual e fio dental manual; *A*: Escova manual e fio dental automático. Os pacientes dos grupos de tratamento *M* e *A* realizaram este regime de higiene durante 1 mês. Após este mês, os pacientes de tratamento trocavam seu grupo de higiene, os que utilizavam fio dental manual passaram a utilizar fio dental automatizado e vice versa. Entre estes dois meses de tratamento houve um período de *washout (14 dias)* em que os pacientes foram orientados a realizar seus hábitos de higiene anteriores ao estudo. Os pacientes do grupo controle continuaram realizando a mesma higiene durante todo

experimento. Orientação de higiene bucal foi realizada no baseline dos dois períodos do estudo. Acúmulo de placa interproximal foi mais pronunciada após intervenção no grupo controle (*C)* do que nos grupos fio dental manual (*M)* e fio dental automático (*A)* na análise de toda a boca. As médias de Índice Gengival (IG) na análise ajustada foram maiores no grupo controle *(C)* do que nos grupos de tratamento *M e A.* Entretanto, significância estatística foi encontrada apenas no grupo *C* em relação ao grupo *A* aos 30 dias. Os autores afirmam que embora a diferença de acúmulo de placa tenha sido estatisticamente significante, estes resultados não necessariamente são clinicamente relevantes, porque a maior diferença encontrada foi de 0,29 no dia 30. A falta de significância estatística entre os grupos para IG pode ser atribuída aos níveis baixos de inflamação gengival no início do estudo.

Em 2008, Rosema et al. realizaram um ensaio clínico randomizado para avaliar se indivíduos com gengivite moderada conseguem manter níveis reduzidos de placa e melhores condições gengivais após um período pré-experimental de 3 semanas utilizando diferentes dispositivos de higiene bucal. No período pré-experimental, os pacientes foram orientados a realizar higiene bucal com escova multicerdas, dentifrício fluoretado e bochechar peróxido de hidrogênio e clorexidina (0,2%). No baseline do período experimental, os pacientes receberam raspagem e polimento nos dentes, foram randomizados em 3 grupos de tratamento (*MB:* Escova multicerdas manual sozinha, *MBF:* Escova multicerdas manual e fio dental e *PB:* Escova motorizada sozinha), e orientados a realizar higiene bucal de acordo com seu regime de tratamento. Os pacientes retornaram à clínica mais duas vezes para reforço de orientação de higiene (após 6 e 10 semanas). Exames clínicos parciais foram realizados nos dias 0 (início do período pré-experimental), baseline, 10 semanas, 6 meses e 9 meses. A condição gengival no baseline não apresentou diferença entre os grupos. No exame clínico de 6 meses, os grupos *MBF* e *PB* foram mais efetivos em manter menores escores de sangramento do que o grupo *MB.* Aos 9 meses esta diferença entre os grupos perde a significância. Entretanto, observa-se que todos os grupos reduziram os níveis de placa e de gengivite ao final do período experimental (9 meses) quando comparado ao dia 0 (anterior ao pré-baseline).

Graziane et al., 2018 avaliaram sessenta pacientes periodontalmente saudáveis em diferentes regimes de tratamento de higiene oral durante 1 mês. No início do estudo, os participantes foram randomizados em 4 grupos: G1: escova multicerdas manual sozinha; G2: Escova multicerdas manual e fio dental; G3: escova

multicerdas manual e escova interdental; G4: escova multicerdas manual e palitos interdentais. Neste mesmo dia, os pacientes receberam orientação de higiene bucal de acordo com o seu grupo de tratamento. Durante o período experimental, os pacientes retornaram a cada duas semanas (dia 14 e 28) para exames clínicos, mas nenhuma orientação de higiene ou intervenção profissional foi realizado. Ao final do período experimental (dia 28), todos os grupos reduziram níveis de placa e gengivite. Já o acúmulo de placa e inflamação gengival interproximal foi menor nos grupos que utilizaram escova interdental e palito interdental quando comparado ao grupo que utilizou apenas a escova multicerdas. O grupo que utilizou fio dental não demonstrou diferença nos níveis de placa e gengivite para sítios interproximais quando comparado a escova multicerdas manual sozinha. Os autores acreditam que o fio dental demanda uma técnica manual complexa que pode interferir no comprometimento dos pacientes. Além disso, os participantes receberam apenas uma orientação de higiene bucal durante todo o período experimental.

Kotsakis et al., 2018 avaliaram a eficácia comparativa de dispositivos de higiene interproximal usando uma Meta-Análise Bayesiana Network (MABN). O desfecho primário foi inflamação gengival mensurado com Índice Gengival (IG) e Sangramento a Sondagem (SS). Placa e profundidade de sondagem (PS) foram considerados desfechos secundários. Setenta e um artigos foram selecionados para leitura completa do texto, apenas vinte e dois preencheram os critérios de inclusão e foram incluídos na meta-análise bayesiana network (MABN). Dez variedades de dispositivos de higiene interproximal foram encontrados nos estudos e avaliados neste meta-análise. Escova interdental e “water jet” são os dispositivos que foram melhor classificados para redução de inflamação gengival. O fio dental sem orientação de higiene não se mostrou eficaz na redução de placa interproximal. Apesar dos resultados desta MABN não ser positivo em relação ao fio dental, os autores não refutam sua prática. Eles acreditam que fio dental é eficaz na remoção de placa e inflamação gengival quando utilizado de forma efetiva. Entretanto, a dificuldade da técnica pode ter dificultado a adesão dos pacientes para este dispositivo.

Em 2019, Worthington et al. conduziram uma revisão sistemática para avaliar a eficácia de dispositivos interdentais associados a escovação comparados com escovação sozinha para prevenção e controle de doenças periodontais, cárie e placa. Quinze estudos que avaliaram fio dental associado a escova manual comparado a escova manual sozinha foram selecionados. Evidências de baixa certeza sugeriram

que o uso do fio dental associado a escova manual reduz gengivite comparado a escova manual sozinha em 1, 3 e 6 meses. A maioria dos estudos desta revisão eram de curto prazo e envolveram pacientes com baixo nível de inflamação gengival. Os autores sugerem que novos ensaios clínicos randomizados de maior tempo de acompanhamento e que relatem as condições periodontais dos pacientes devem ser realizados para responder se dispositivos interdentais associado a escovação tem efeito nas condições gengivais.

Tabela 1- Revisão de Literatura

Autor	N	Condição gengival inicial	Grupos, foi randomizado?	Orientação de Higiene Bucal (OHB)	Intervenção Profissional	Tempo do estudo	Desfechos	Resultados
Vogel et al 1975	24	Pacientes saudáveis. (Estudantes de odontologia).	ECR 4 grupos: G1: escova (E); G2: E+fio (F); G3: E+palito (P); G4: E+F+P	Período pré-experimental e durante os dias de exame (0, 9, 15 e 33 dias)	No Período pré-experimental e nas datas de exames.	33 dias	IG;	4 grupos apresentaram saúde gengival ao final do estudo. Sem diferença entre grupos.
Kiger et al. 1991	30	Pacientes com periodontite tratados.	Cross-Over; Randomizado 3 Grupos: G1: E; G2: E+F G3: E+escova interdental (EI);	1 vez por mês. A cada mês para uso de um dispositivo diferente.	Sim. OHB e profilaxia uma vez por mês	3 meses	QHI; IG;	E+EI foi mais efetivo para remoção de placa interproximal do que E sozinha ou em combinação com F.
Bauroth et al., 2003	362	Gengivite leve a moderada	ECR; 3 grupos: E+colutório; E+F; E+colutório com óleos essenciais;	Baseline	Apenas no baseline.	6 meses	MGI;	Em 6 meses, houve diferença estatística para MGI interproximal entre os grupos. Média (DP) de MGI interproximal foi de 2.05(0.24) para E+colutório; 1.92 (0.22) para E+F; 1.79 (0.31) para E+colutórios óleos essenciais.

Zimmer et al., 2006	156	Gengivite; NIC < 5mm;	ECR 4 grupos: E+colutório clorexidina 0.06%; E+colutório cloreto de cetilperidínio 0.1% + 0.025% flúor;; E+F; E.	Pacientes receberam breve instruções de como utilizar escova, fio dental e colutórios. Instrução para fio dental foi realizada em modelo plástico em tempo máximo de 2 min.	Não	8 semanas	QHI; MPPI; PBI;	Nenhuma diferença foi observada entre E versus E+F para qualquer parâmetro avaliado.
Schiff 2006	114	Gengivite (Média de IG=1);	ECR; 3 grupos: E (Dentifrício fluoretado com triclosan)+F; E (Dentifrício fluoretado com triclosan); E (Dentifrício fluoretado)+F.	Somente no dia da randomização.	Profilaxia apenas no baseline.	6 meses	QHI; IG;	Grupo E (Dentifrício fluoretado com triclosan) apresentou menores valores médios de IG quando comparado ao E (Dentifrício fluoretado com triclosan)+F, mas sem diferença estatística. Grupos com dentifríco contendo triclosan promoveram redução estatisticamente maior de IG quando comparado do grupo E+F.
Hague e Carr 2007	102	Gengivite Leve	ECR Crossover. 3 grupos: *E (C:* Controle); E+F manual; E+F automatizado	Baseline e mais duas vezes durante o estudo.	Não	10 semanas	QHI: IG:	O grupo controle (*C)* apresentou maiores medias de acúmulo de placa comparado aos grupos fio dental manual *(M)* e fio dental automático *(A).* Inflamação gengival após

								análise ajustada apresentou maiores níveis de IG no grupo *C* comparado aos grupos *M* e *A.* Entretanto, diferença estatisticamente significante foi encontrada apenas no grupo *C* comparado ao grupo *A* aos 30 dias.
Rosema et al. 2008	118	Gengivite moderada; Sem perda de inserção;	ECR 3 grupos: E; E+F; E Motorizada.	Período pré-experimental, baseline e após 3 semanas.	OHB nos primeiros 3 meses a cada 3 semanas	9 meses	BOMP;	Sem diferenças estatísticas entre os grupos em 9 meses. Em 6 meses, E motorizada apresentou menores médias de sangramento quando comparada aos grupos E+F e E, os quais não diferiram entre si.
Graziani et al 2018	60	Sem perda de inserção.	ECR 4 grupos: E; E+F; E+EI; E+P de borracha	1 vez durante o período pré-experimental.	Profilaxia no baseline do período experimental.	4 semanas	FMPS; FMBS; AngBI;	Após 28 dias, nenhuma diferença estatística foi observada entre os grupos para escores de placa ou sangramento full-mouth. Em sítios proximais, os grupos E+EI e E+P apresentaram menores médias de placa e diferença estatística em relação ao grupo E.

AngBI: Índice de Sangramento Angulado (VAN DER WEIJDEN et al., 1994); BOMP: Índice de Sangramento a Sondagem Marginal (LIE et al., 1998); ECR: Ensaio Clínico Randomizado; E: escova multicerdas; EI: escova interdental; F: fio dental; FMPS: Escore de Placa toda boca (O'LEARY; DRAKE; NAYLOR, 1972); FMBS: Escore de Sangramento toda boca (AINAMO; BAY, 1975); IG: Índice Gengival (LÖE 1965); IP: Índice de Placa (SILNESS; LÖE, 1964); MGI: Modified Gingival Index (LOBENE et al., 1986); MPPI: Índice de Placa Proximal Modificado (LANGE et al., 1977); OHB: Orientação de Higiene Bucal; P: palito; PBI: Índice de Sangramento Papilar (SAXER; MÜHLEMANN, 1975); QHI: Índice de Placa modificado por Quigley-Hein (TURESKY et al., 1970);

ARTIGO 1– **TOOTHBRUSHING SUPPLEMENTED BY DENTAL FLOSS IS MORE EFFECTIVE TO REDUCE GINGIVITIS THAN TOOTHBRUSHING ALONE IN SUBJECTS PERFORMING PROPER TECHNIQUE OF ORAL HYGIENE**

Este artigo será submetido ao periódico *Journal of Clinical Periodontology* , ISSN: 1600-051X, Fator de impacto: 5.241, Qualis A1. As normas para publicação estão escritas no Anexo A.

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TOOTHBRUSHING SUPPLEMENTED BY DENTAL FLOSS IS MORE EFFECTIVE TO REDUCE GINGIVITIS THAN TOOTHBRUSHING ALONE IN SUBJECTS PERFORMING PROPER TECHNIQUE OF ORAL HYGIENE

Running Title: Flossing reduces interproximal gingivitis

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FUNDING INFORMATION: This study was partially supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this study.

CLINICAL TRIALS: ClinicalTrials.gov: (53831716.5.0000.5346)

Author contribution: All authors met the four criteria stablished by ICMJE definition of authorship, which included (i) substantial contributions to the conception of the work, or the acquisition, analysis, or interpretation of data; (ii) drafting the work; (iii) final approval do the version to be published; (iiii) agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data Availability Statement: The data that support the findings of this study are openly available in Londero et al., 2021.

ABSTRACT

Aims: to evaluate, (i) if subjects submitted to an oral hygiene (OH) training would be able to maintain the learned habits during 180-days without professional supervision; (ii) if dental floss provide additional benefits to the toothbrushing on gingival health.

Material and Methods: 75 adults subjects showed about 40% of full-mouth proximal gingival bleeding were randomized to receive OH training (8 sessions once a week) with toothbrush (TB) or toothbrush plus dental floss (TB+). After, subjects were followed for 180-days without professional intervention. Primary outcomes were interproximal Gingival Index(GI) and GI-score=2 (gingival bleeding) means. Mixed linear models were used for the analysis and comparison between groups ($p<0.05$).

Results: 68 subjects completed the OH training, and 48 completed the follow-up of 180-days. Follow the gingival inflammation reduction associated with the training period, no alteration in the gingival status was observed into the groups during 180-days showing that subjects maintained OH adequate

behavior. TB+ (12.8±2.5) showed lower statistically mean of GI=2 interproximal sites when compared to the TB (19.8±2.2) at 180-days.

Conclusions: When patients are trained to proper flossing, dental floss associated with toothbrushing promote higher interproximal gingival inflammation reduction when compared to the toothbrushing alone. ClinicalTrials.gov: (53831716.5.0000.5346)

Keywords: dental floss; gingival disease; gingivitis; oral hygiene;

CLINICAL RELEVANCE:

Scientific rationale for study: Effective personal oral hygiene procedures is essential to maintain oral health. The dental floss importance on the gingival health needs be clarified since the scientific evidences that support the dental floss recommendation to reduce gingivitis present low-certainty evidence.

Principal findings: In patients trained to proper flossing, toothbrushing supplemented by dental floss promote higher interproximal gingivitis reduction when compared to the toothbrushing alone even after 180-days without professional supervision.

Practical implications: dentists need to invest time in training/motivating their patients to achieve adequate OH; adjunct flossing in subjects with papilla filling the interdental space is essential to reach gingival health.

INTRODUCTION

There is a common sense among dentists, that oral hygiene (OH) should include toothbrushing and some device to remove dental plaque specifically on interproximal surfaces. Flossing has been indicated once a day as essential part of the OH routines for sites where the interdental space did not allow the passage of an interdental brush (American Dental Association, European Federation of Periodontology, American Academy of Periodontology).

However, despite public knowledge that flossing is necessary to maintain gingival health, the literature report high prevalence of gingivitis and periodontitis in different populations, especially on interproximal surfaces (Li et al., 2010). This means that or the people did not use the dental floss or they use unproperly. Some evidences have reported that poor adherence to the dental floss use is associated with technique difficulties (Ethan & Lim, 2019), especially in posterior molars regions (Santos 2003; Hague & Carr, 2007), and with lack of motivation to perform its use daily (Asadoorian & Locker 2006). Recent network meta-analysis (Kotsakis et al., 2018) states that dental floss without proper professional instruction does not reduce gingival inflammation. In contrast, when performed adequately, flossing has efficacy potential to prevent caries and gingivitis. Daily professional flossing prevented the incidence of caries in schoolchildren by 40% (Hujoel, Cunha-Cruz, Banting, & Loesche 2006). Therefore, seems logical that if we want our patients to use dental floss properly, we will have to train and motivate them to do it in their daily routine.

Previous randomized clinical trials have failed show additional effect of the dental floss as a supplement to toothbrushing on gingival inflammation reduction (Graziani et al., 2018; Zimmer et al., 2006; Hague & Carr, 2007). Some systematic reviews (Berchier et al., 2008; Sambunjak et al., 2011; Sälzer et al., 2015; Kotsakis et al., 2018; Worthington et al., 2019) and a network metanalysis (Kotsakis et al., 2018) have questioned the scientific evidence that support the dental floss recommendation to reduce gingival inflammation. The authors have indicated low-certainty evidence and that the effect sizes may not be clinically important (Kotsakis et al. 2018; Worthington et al. 2019).

In fact, most of the previous studies did not performed an essential training to help participants to learned proper dental floss technique. Some studies performed OH instruction once (Bauroth et al., 2003; Zimmer et al., 2006; Hague & Carr, 2007; Graziani et al., 2018). In the best our knowledge, the present study is the first report of subjects submitted to an OH training including toothbrushing and flossing (weekly 8 sessions) followed-up during 6 months with no professional interference.

The current study is an analysis of follow up of a randomized parallel-group clinical trial, which the subjects were randomized to perform their OH with toothbrushing or toothbrushing plus flossing. These subjects received weekly 8 sessions of oral hygiene instruction (OHI), which they performed complete plaque removal supervised by a researcher. In this previous report (Londero et al., 2021), we demonstrated that the interproximal gingival inflammation may be significantly reduced by toothbrushing alone and toothbrushing supplemented by dental floss, but the effect was significantly greater when toothbrushing was combined with dental floss. Here, we show the what happened with the gingival status of the same subjects after 180 days without any professional interference or OH instructions. We consider two questions: (i) Would subjects be able to maintain the learned OH habits for longer periods of time without any professional supervision? (ii) Would the adjunct use of dental floss continue to provide additional benefits to toothbrushing, as observed during the training period even if subjects were left without professional supervision? Our initial hypothesis was that subjects submitted to the OH training would be able to maintain adequate OH technique over time even in the dental floss group.

MATERIALS AND METHODS

Trial design

The present study is an analysis of follow up of a randomized parallel-group clinical trial previously published (Londero et al., 2021).

Participants and sample size

Subjects were invited to participate through the Federal University of Santa Maria screening service, social media and public posters. Eligible subjects should be a minimum age of 18 years, absence of proximal clinical attachment loss, with the intact papilla and at least 24 teeth. Gingival status to eligibility was more than 15% of interproximal sites with gingival bleeding evaluated according the Gingival Index (GI) score 2 (Löe 1967). Posterior distal sites were excluded because they usually are not subject to flossing.

Dental students, diabetics, smokers, subjects with xerostomia, pregnant women, subjects with orthodontic appliances and/or restraint, subjects requiring antimicrobial prophylaxis to perform oral exams; subjects having used antibiotics / anti-inflammatory drugs within the last three months, and subjects with psychomotor disorders were not included.

The sample size calculation considered: GI means in the toothbrushing group and in the toothbrushing plus flossing group of 0.59 and 0.40 and standard deviation of 0.31 and 0.19 (Rosema et al. 2008), respectively; 1:1 ratio in the sample size, 95% confidence interval, 80% test power. A sample of 58 subjects was estimated. We added 30% due to-follow up losses, resulting in a sample estimate of 76 subjects.

The study was conducted June 2017 through August 2020. All the procedures were performed in the Dental School at the Federal University of Santa Maria, Brazil. Eligible subjects were informed about the objectives of the study and those willing to participate signed an informed consent form.

Randomization and Experimental Groups

The block randomization sequence (10 units/block) was generated by a computer program (Random Allocation Software, version 2.0). Allocation and conceal of the random sequence were obtained using sealed opaque envelopes numbered 1 to 76, which corresponded to the sequence from the first to the last participant to be randomized. The randomization procedures were performed by

technical staff. The subjects were randomized in two experimental groups according to OH procedures: toothbrushing plus flossing (TB+ group); and toothbrushing alone (TB group).

OH training period

Supragingival scaling, reshape of restorations, sealing of cavities were performed according to individual needs using 1 or 2 therapeutic sessions. OH training was performed by 8 sessions once a week which they performed complete plaque removal supervised by a researcher during 60 days. After to reveal the experimental group to which the participant belongs, the clinical staff member (A.B.L.) performed the OH instruction. Each OH instruction session followed a sequence of standardized procedures. First, the researcher demonstrated, in the subject mouth how brushing should be performed, while the subjects accompanied the demonstration in front of a mirror. Subjects from TB+ group also received orientation of flossing on all proximal surfaces. Second, the subject was asked to perform their oral hygiene in front of the mirror and the researcher corrected the inadequacies. At the end, the subjects were oriented to perform their OH at home following the trained technique performing toothbrushing twice a day (TB and TB+ groups) and using flossing (TB+ group) once a day (Chapple et al., 2015; ADA 2019).

Participants received a soft toothbrush (Colgate® Twister® Compact Head, New York, USA) and fluoride dentifrice (Colgate® Triple Action®, 90 grams, New York, USA). Subjects belonging to the TB+ group received a waxed dental floss box (Colgate®, tarpaulin, New York, USA).

Experimental Period

After the OH training period, no further interventions were carried out. Subjects were instructed to perform their OH following the technique and frequency recommended. Clinical examination was performed at the randomization day, at the end of the training period, and after 60 and 180 days. Toothbrush and dental floss were replaced at 60 days.

Adhesion measures to the OH procedures performed at home were evaluated from questionary applied at 60 and 180 days. Ask about the daily frequency of toothbrushing and flossing were answered by subjects.

The Figure 1 shows the time line of the study.

Clinical Parameters

Clinical parameters of Plaque Index (PI) (Silness & Löe, 1964), GI (Löe 1967), probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BoP) were evaluated at six sites per tooth (mesiobuccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual) using a millimeter periodontal probe (North Carolina, Neumar®, São Paulo, Brazil), with the exception of the third molars. The distal site of the most posterior teeth of the arch were not evaluated.

Clinical parameters were measured by two blinded examiners. Prior to the start of the study, the examiners participated of a training conducted by experiment examiner (C.H.C.M) with clinical discussion on each score or category and on possible disagreements for PI, GI, CAL, PD and BoP. Training was finished when an adequate level of agreement and understanding of the parameters was reached. R.C.R.T examiner was calibrated prior to data collection for PD and CAL assessments reaching intraclass correlation coefficient of 0.78 and 1, respectively. Training procedures were carried out in subjects not include in the study.

Outcomes

Primary outcome was the proximal gingival inflammation measured from mean of GI=2 and of GI. Secondary outcome was the interproximal plaque accumulation evaluated from mean of PlI=2 and of PlI.

Ethical Considerations:

Eligible subjects provided informed consent. This study was conducted by following the Guidelines and Norms Regulating Research involving humans. The research protocol was submitted and approved by the Research Ethics Committee of the Federal University of Santa Maria (UFSM) (CAAE: 53831716.5.0000.5346).

Statistical Analysis

Analysis data was carried out using the Statistical Package for Social Science (SPSS for Windows, version 21.0, SPSS Inc.). Subject was the unit analysis. Summary statistics included mean and standard deviation of PI, GI, PD, CAL, BoP and GI=2. Data were analyzed by intention to treat and by protocol. Multiple imputation was used to replace missing data. Linear regression was used to impute missing data, with time and group being used as predictors. Twenty imputations were run in order to better converge on strong approximations of missing data (Leech, Barrett, & Morgan, 2015). Mixed linear models were used for the analysis and comparison of groups. The best covariance structure (component symmetry) was tested. The parameters of the model were estimated through maximum probability. The level of significance was set at 5%

RESULTS

Seventy-five subjects were randomized, and sixty-eight did complete the OH training period. Table 1 shows the clinical, demographic and behavioral characteristics of the 75 subjects at randomization time and of the 68 subjects that completed the OH training and started the follow-up period. No statistical difference was observed between groups to any parameter and any time. At randomization time, full-mouth mean of sites with gingival bleeding (GI=2) was approximately 38% in both, TB and TB+ groups. After the OH training, the mean was reduced to 21% and 16% to TB and TB+ groups, respectively. Specifically in interproximal sites, the subjects presented 41.0% and 37.7% of GI=2 in the randomization time, reducing to 22.3% and 15.6% after the training period to TB and TB+, respectively.

Twenty individuals did not complete the 6-month (180-day) follow-up period, ten participants from each group. The main reason of the losses was the covid-19 pandemic in Brazil. There was social withdrawal and the Federal University of Santa Maria was closed; some participants reported family disease associated with covid-19; and some participants did not answer our calls (Figure 2). Table 2 presents the characteristics of the 48 subjects that remained in the study until the clinical examination of 180 days in the moment of the start of follow-up period. The data are showed comparing the characteristics of drop-out and non-drop-out subjects according the experimental group. No statistical difference was observed for any parameter evaluated between TB (n=27) and TB+ (n=21) groups. In the TB+ group, no statistical difference was observed for any parameter between lost subjects and those finalized the study. However, in the TB group, subjects drop-out (n=10) showed statistically higher mean of GI=2, BoP and PD when compared to the subjects that completed the follow-up of 180 days (n=27).

Due the dropouts, we performed a new calculation to verify the power of our study considering: a clinically relevant difference of 10% in the percentage of GI=2 sites between groups, standard deviation of 11.2 and 12.7 for TB and TB+, respectively, which were the SD observed at 180 days, the sample of 48 subjects, who completed the follow-up of 180 days. The calculation showed a power of 80.6%.

Table 3 shows proximal gingival inflammation measured from mean of GI=2 and of GI analyzed by protocol. Intention-to-treat analysis was also performed presenting similar results (Appendix A). Subjects of the TB group started the follow-up period with higher statistically mean percentage of sites GI=2 when compared to the TB+ subjects, consequence of the training period. Both groups, TB and TB+, maintained GI=2 mean over time, even without professional supervision. At 180 days, TB+ group presented a lower statistically mean percentage of sites GI=2 when compared to the TB group. Regarding GI mean, both the groups reduced the mean values through study, but only in the TB+ the reduction was statistically significant.

In both the groups, TB and TB+, the mean values of PI=2 (visible plaque) and of PlI increased through study, but without statistical significance. No difference was observed between groups (Table 4).

Approximately 40% of the subjects in the TB and TB+ groups answered performed toothbrushing twice a day. Independently of the group, most of the subjects reported performed toothbrushing 3 times a day (Table 5). In the TB+ group (n=21), 15 and 16 subjects reported to use dental floss once a day at 60 and 180 days, respectively. Two participants reported that did not daily adhered to the dental floss use (Table 5).

DISCUSSION

In previous report (Londero et al., 2021), we demonstrated that flossing in addition to toothbrushing reduce interproximal gingival inflammation more than toothbrushing alone during a period of OH training. In the present study, we consider two questions: (i) Would subjects be able to maintain the OH learned technique over longer periods of time without any professional supervision? (ii) Would the adjunct use of dental floss continue to provide additional benefits to the toothbrushing, as observed during the training period even if subjects were left without professional supervision? First, our findings showed that subjects maintained OH adequate behavior during 6 months without professional supervision in both groups, TB and TB+, since no alterations in the gingival status was observed into the groups over time. Second, the adjunct use of dental floss maintained the additional benefits to the toothbrushing, since TB+ group (12.8±2.5) showed a lower statistically mean percentage of proximal sites GI=2 when compared to the TB (19.8±2.2) at 180 days.

No plaque accumulation change was verified over 180 days, and no statistical difference was observed between groups, despite TB+ group to show lower statistically proximal GI=2 mean when compared to the TB. Notwithstanding classic studies have established the etiological relationship between bacterial accumulations and initiation/reversion of gingivitis (Lang, Cumming, & Löe, 1973; Loe, Theilade, & Jensen, 1965), the correlation between clinical plaque scores and gingival scores is not direct (Danielsen et al., 1989; Lie et al., 1998; De David et al., 2018; Reiniger et al., 2021). The reasons

for it include the fact that plaque is evaluated only from quantitative measures, while its composition is not considered. Besides, other factors such as personal profile on immune inflammatory response (Trombelli et al., 2004) can biases the correlation among these parameters.

Our findings showed that eight weeks of OH training delivered from periodontist was enough to subjects modify and maintain better habits of OH during 6 months, even in subjects performing flossing. This is of particular interest considering that the use dental floss is not easy, and it is necessary training for removing dental plaque on proximal areas. It has been shown that flossing effectiveness decreases in the absence of frequent reinforcement and instruction (Stewart & Wolfe, 1989), and that the motivation to floss decreases as the time since the last dental visit increases (Macgregor, Balding, & Regis, 1998). Here, the subjects in the TB+ group, not only maintained their gingival status, but reduced statistically the GI mean between the start of the follow-up period and 180 days. Caution must be exercised when interpretations are made from our findings considering that 180 days is a short time period to evaluate the effectiveness of a habit change associated with oral health (Raj et al., 2013). Therefore, the typical recall interval of 6 months between the professional visits has demonstrated to be effective to maintain the periodontal health (Axelsson, Nyström, & Lindhe 2004). In previous study (Rosema et al., 2008), subjects that received four OH with toothbrush alone or toothbrush supplemented by dental floss demonstrated an increase in gingival bleeding between 6 and 9 months (Rosema et al., 2008).

Previous clinical trials also demonstrated that flossing plus toothbrush promote higher gingival inflammation reduction when compared toothbrush alone at 6 months (Sharma et al. 2002; Bauroth et al. 2003; Rosema et al. 2008). However, methodological differences between our study and aforementioned studies (Sharma et al., 2002; Bauroth et al., 2003; Rosema et al., 2008) restrain direct comparisons. Among these differences, we highlight the period of OH training (weekly 8 session), and the subjects initial gingival status included in the training, who should present high level of proximal gingival inflammation indicating an inadequate OH behavior. In the Bauroth study, 2003, the flossing group subjects were instructed in the proper use of dental floss once, and the subjects had a low level of proximal gingival inflammation before received the instruction. This means that the sample showed

adequate OH habits before of start the study. In another previous study (Rosema et al., 2008), the participants had a level of gingival bleeding > 40% at baseline. The participants were submitted pre-experimental phase, which subjects had not been assigned to their specific oral hygiene group and all subjects used chlorhexidine mouthwash twice a day. This means that the gingival inflammation reduction reached during this period was not only associated with the subjects learned about the toothbrush or dental floss technique, but also with chlorhexidine effect. Recent Cochrane systematic review (Worthington et al., 2019) indicated dental floss in addition to toothbrushing may reduce gingivitis more than toothbrushing alone; although, outcomes have been measured in the short term and participants in most studies have showed a gingival inflammation low level at baseline. In the present study, we included subjects that presented before OH training 41.0% and 37.7% of proximal gingival bleeding characterizing a high level of inflammation in the TB and TB+ groups, respectively. This subject's initial condition was an important strength of the present study because indicated that subjects did not know or did not have motivation to preform proper OH in their daily routine. Therefore, we can evaluate if subjects with gingivitis learned and maintained new behavior over 180 days. The dropouts associated with the covid-19 pandemic restrained to follow the subjects for a longer period of time.

Our study has limitations. Regarding dropouts during the follow up of 180 days some aspects must be discussed: (i) the dropouts were not associated with the oral hygiene procedures type, but with the covid-19 pandemic. (ii) We verified that the dropouts did not affect the comparability between groups. No significant statistically difference was verified between TB and TB+ groups in the start of follow up when we compared only the subjects that finalized the 180 days. (iii) However, when we pay attention into of each group, we verified that no difference was observed between drop out and not drop out subjects in the TB+ group; but, in TB group, subjects that dropped out showed higher statistically mean of GI=2 for both, full-mouth and interproximal sites. This means that the subjects with worst gingival inflammatory status dropped out into TB group. Therefore, the effect the self-performed OH on the gingival inflammation reduction in the TB group can have been overestimated. (iiii) As 20 subjects, 10 in each group dropped out, we were concerned about the power of our study. Then, we carried out a new calculation considering a clinically relevant a difference of 10% in the percentage of

GI=2 sites between groups and a sample of 48 subjects, who completed the follow-up of 180 days. The calculation showed a power of 80.6% reaching a level commonly applied in research.

Another important aspect in our study was to assess participants adhesion to the OH procedures that they should perform at home without supervision during 180 days. Interestingly, the common popular sense of performed toothbrushing three times a day remained independently of our recommendations of toothbrushing daily frequency of twice a day. As this was verified in most of the subjects in both groups, TB and TB+, we consider there was not bias to detect the differences in the effect estimate between groups. Dental floss adhesion was high considering that more than 70% of the subjects reported daily flossing as recommended; and about 19% and 15% of the subjects reported dental floss use twice a day at 60 days and 180 days, respectively. Two participants of the TB+ group reported that did not use daily dental floss. Therefore, some bias in the dental floss performance to reduce gingival inflammation may have occurred.

The mean percentage of proximal sites showing gingival bleeding after the training period (22.3% and 15.6%), and after the follow-up (19.8% and 12.9%) in TB and TB+, respectively, was not enough to reach the cut off stablished in 2018 from current Classification of Periodontal and Peri-Implant Disease and Conditions (Lang & Bartold, 2018) that define gingival health as a BoP score <10%. Here, we did not evaluated BoP. However, considering that the subjects presented minimal attachment loss and shallow PDs, it's possible exercise that BoP and GI=2 indicate inflammatory alterations in the marginal gingiva, since positive correlation was verified between these parameters in a sample with these clinical status (Reiniger et al., 2021). In this point, why did the individuals not reach the cut-off point established to define gingival health? First, it's clear that toothbrushing alone did not even come close of reach health gingival, indicating that the floss is essential for it in proximal surfaces. Second, TB+ group subjects started the training period with about 38% of bleeding proximal sites; they reduced to 15.6% after the OH training, and then, they showed an additional reduction in 180 days, even without professional supervision (12.9%). It's possible that TB+ group did not reach gingival health because the cut off of gingival bleeding <10% was very low considering the they had a high level of baseline gingival inflammation.

To prevent/arrest gingivitis/periodontitis, and dental caries, it's essential to establish lifelong effective personal oral hygiene measures achieved through a balance of oral hygiene events and their temporal recurrence (Axelsson, Nyström, & Lindhe, 2004). Considering this context, the findings that OH training promotes proximal gingival inflammation reduction and maintenance of this result over 180 days, introduce important clinical interpretations: (i) dentists need to invest more time in training and motivating their patients to obtain adequate self-control of plaque, regardless of the devices indicated for it; and (ii) adjunct dental floss to the toothbrushing in subjects with papilla filling the interdental space, is essential if the aim is to reduce gingival inflammation to parameters close to those established by new classification (2018) to define gingival health.

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Table 1 – Sociodemographic, behavioral and clinical characteristics of the 75 subjects randomized and of the 68 subjects available to start follow-up period

Parameter		**Randomization n=75**			**Start of follow-up n=68**		
		TB n= 38	**TB+ n=37**	**P**	**TB n= 37**	**TB+ n=31**	**P**
Age ($\bar{x}$ ± sd)* years		23.97 (5.5)	22.32 (7.6)	*0.29*	24.09 (5.5)	22.19 (8.1)	0.28
Gender n (%)#	Female	16 (42.1)	24 (64.9)		16 (43.2)	19 (61.3)	
	Male	22 (57.9)	13 (35.1)		21 (56.8)	12 (38.7)	
Socioeconomic level n (%)#							
Family monthly income	≤1 Brazilian minimum wage	9 (24.3)	6 (16.7)	*0.56*	9 (25.0)	6 (20.0)	0.43
	≥2 Brazilian minimum wage	28 (75.7)	30 (83.3)		27 (75.0)	24 (80.0)	
Educational level (years)	≤ 12 years	7 (23.3)	8 (28.6)	*0.77*	7 (24.1)	6 (25.0)	0.60
	> 12 years	23 (76.7)	20 (71.4)		22 (75.9)	18 (75.0)	
Behavioral n (%)#							
Daily frequency of toothbrushing	Up to two	16 (42.1)	17 (45.9)	*0.82*	15 (40.5)	15 (48.4)	0.34
	≥ 3 times	22 (57.9)	20 (54.1)		22 (59.5)	16 (51.6)	
Interdental device use	No	14 (36.8)	13 (35.1)	*0.99*	14 (37.8)	10 (32.3)	0.41
	Yes	24 (63.2)	24 (64.9)		23 (62.2)	21 (67.7)	
Full-mouth clinical parameters ($\bar{x}$ ± sd)*							
PlI		0.66 (0.4)	0.70 (0.4)	*0.74*	0.45 (0.4)	0.48 (0.4)	0.79
GI		1.38 (0.1)	1.34 (0.2)	0.37	1.13 (0.2)	1.09 (0.2)	0.40
GI=2		39.42 (15.6)	36.01 (16.9)	0.37	21.40 (14.7)	16.18 (12.8)	0.12
PD (mm)		1.74 (0.2)	1.64 (0.3)	0.15	1.61 (0.2)	1.54 (0.2)	0.25
BoP (%)		23.10 (10.9)	21.95 (13.7)	0.69	15.49 (13.3)	12.62 (8.3)	0.28
CAL (mm)		0.05 (0.09)	0.04 (0.06)	0.56	0.07 (0.10)	0.07 (0.06)	0.89

*mean and standard deviation.
distribution frequency.
Abbreviations: TB: toothbrush group; TB+: dental floss group; PlI: Plaque Index (Silness & Löe, 1964); GI: Gingival Index (Löe & Silness 1963, modified by Löe 1967); PD: probing depth; BoP: bleeding on probing; CAL: clinical attachment level.

Table 2. Sociodemographic, behavioral and clinical characteristics comparing the drop-out and non-drop-out at baseline (start of the follow-up period).

Parameters		**Non-drop-out (n=48)**	**Drop-out (n=20)**	**P**
Age ($\bar{x}$ ± sd)* years		24.27 (5.70)	22.60 (5.69)	0.28
	TB	24.67 (5.83)	22.70 (4.47)	0.28
	TB+	23.76 (5.64)	22.50 (6.95)	0.62
		0.59		
Gender n (%)[#]				
Female		23 (47.9)	9 (45.0)	0.52
	TB	11 (40.7)	2 (20.0)	0.44
	TB+	12 (57.1)	7 (70.0)	0.70
		0.38		
Male		25 (52.1)	11 (55.0)	
	TB	16 (59.3)	8 (80.0)	0.44
	TB+	9 (42.9)	3 (30.0)	0.70
		0.38		
Clinical parameters ($\bar{x}$ ± sd)*				
PIl		0.41 (0.32)	0.60 (0.45)	0.10
	TB	0.38 (0.24)	0.67 (0.53)	0.12
	TB+	0.45 (0.40)	0.53 (0.38)	0.62
		0.21		
GI		1.13 (0.17)	1.08 (0.28)	0.46
	TB	1.13 (0.17)	1.14 (0.33)	0.92
	TB+	1.12 (0.18)	1.02 (0.22)	0.20
		0.86		
GI=2 (%)		17.18 (12.23)	23.46 (17.21)	0.15
	TB	17.08 (11.00)	33.09 (17.55)	0.02
	TB+	17.31 (13.93)	13.82 (10.51)	0.49
		0.38		
Interproximal GI=2 (%)		17.11 (11.88)	24.39 (18.36)	0.11
	TB	17.87 (10.85)	34.37 (18.52)	0.02
	TB+	16.15 (13.31)	14.40 (12.12)	0.72
		0.86		
PD (mm)		1.53 (0.21)	1.71 (0.29)	0.02
	TB	1.54 (0.19)	1.83 (0.30)	0.02
	TB+	1.51 (0.24)	1.61 (0.24)	0.34
		0.32		
BoP (%)		12.04 (7.74)	19.29 (16.21)	0.07
	TB	10.38 (6.29)	29.23 (17.45)	0.02
	TB+	14.17 (8.99)	9.36 (5.60)	0.13
		0.23		
CAL (mm)		0.08 (0.09)	0.05 (0.06)	0.11

TB	0.08 (0.10)	0.05 (0.08)	0.43
TB+	0.08 (0.07)	0.05 (0.04)	0.13
	0.70		

*mean and standard deviation.
distribution frequency.
P<0.05 represents statistical difference between groups in the line.
Abbreviations: TB: toothbrushing group; TB+: toothbrushing supplemented by dental floss group; PlI: Plaque Index (Silness & Löe, 1964); GI: Gingival Index (Löe & Silness 1963, modified by Löe 1967); PD: probing depth; BoP: bleeding on probing; CAL: clinical attachment level.

Table 3 – Mean percentage of sites with interproximal gingival bleeding (GI=2) (standard error) and interproximal Gingival Index mean (standard error) during the experimental period, for each experimental group

		TB	**TB+**
GI=2	*Start of Follow up*	22.33 (2.28)^{A,a}	15.59 (2.49)^{B,a}
	60 days	19.67 (2.08)^{A,a}	15.76 (2.39)^{A,a}
	180 days	19.82 (2.23)^{A,a}	12.89 (2.52)^{B,a}
GI	*Start of Follow up*	1.15 (0.03)^{C,c}	1.10 (0.04)^{C,c}
	60 days	1.13 (0.03)^{C,c}	1.11 (0.04)C^{,c}
	180 days	1.06 (0.06)^{C,c}	0.91 (0.07)^{C,d}

TB: toothbrushing group; TB+: toothbrushing supplemented by dental floss group.
Number of subjects at follow up beginning: 37 and 31 for TB and TB+, respectively.
Number of subjects at 60 and 180 days: 27 and 21 for TB and TB+, respectively.
Mixed linear models' analysis
Different uppercase letters (A, B) demonstrate intergroup differences regarding GI=2 (P<0.05)
Different lowercase letters (a, b) show intragroup differences regarding GI=2 (P<0.05)
Different uppercase letters (C, D) demonstrate intergroup differences regarding GI mean (P<0.05)
Different lowercase letters (c, d) show intragroup differences regarding GI mean (P<0.05)

Table 4 – Mean percentage of sites with interproximal PlI (PI=2) (standard error) and interproximal Plaque Index mean (standard error) during the experimental period, for each experimental group

		TB	**TB+**
PlI=2	*Start of Follow up*	11.89(2.72)^{A,a}	12.87(2.97)^{A,a}
	60 days	11.24(2.15)^{A,a}	12.29(2.48)^{A,a}
	180 days	16.15(2.95)^{A,a}	16.57(3.31)^{A,a}
PlI	*Start of Follow up*	0.49(0.06)^{C,c}	0.51(0.07)^{C,c}
	60 days	0.44(0.06)^{C,c}	0.48(0.07)^{C,c}
	180 days	0.51(0.07)^{C,c}	0.51(0.08)^{C,c}

TB: toothbrushing group; TB+: toothbrushing supplemented by dental floss group.
Number of subjects at follow up beginning: 37 and 31 for TB and TB+, respectively.
Number of subjects at 60 and 180 days: 27 and 21 for TB and TB+, respectively.
Mixed linear models' analysis,
Different uppercase letters (A, B) demonstrate intergroup differences regarding PI=2 (P<0.05)
Different lowercase letters (a, b) show intragroup differences regarding PI=2 (P<0.05)
Different uppercase letters (C, D) demonstrate intergroup differences regarding PlI mean (P<0.05)

Different lowercase letters (c, d) show intragroup differences regarding PlI mean (P<0.05)

Table 5 Answers to the adhesion questionary about the oral hygiene procedures performed at home without supervision during the follow up

	Daily frequency of toothbrushing			
	60 days		180 days	
	TB n=27	TB+ n=21	TB n=27	TB+ n=21
1x	0	0	0	0
2x	11 (40.8%)	9 (42.8%)	10 (37%)	9 (42.8%)
3x	12(44.4%)	11 (52.5%)	13(48.2%)	11 (52.5%)
4x	1 (3.7%)	1 (4.7%)	1 (3,7%)	1 (4.7%)
Non-responders	3 (11.1%)	0	3 (11.1%)	0
	Daily frequency of dental floss			
	60 days		180 days	
	TB+ n=21		TB+ n=21	
0x	2 (9.5%)		2 (9.5%)	
1x	15 (71.5%)		16 (76.2%)	
2x	4 (19%)		3 (14.3%)	
Non-responders	0		0	

TB: toothbrushing group; TB+: toothbrushing supplemented by dental floss group.

Figure 1 - Time line of the study

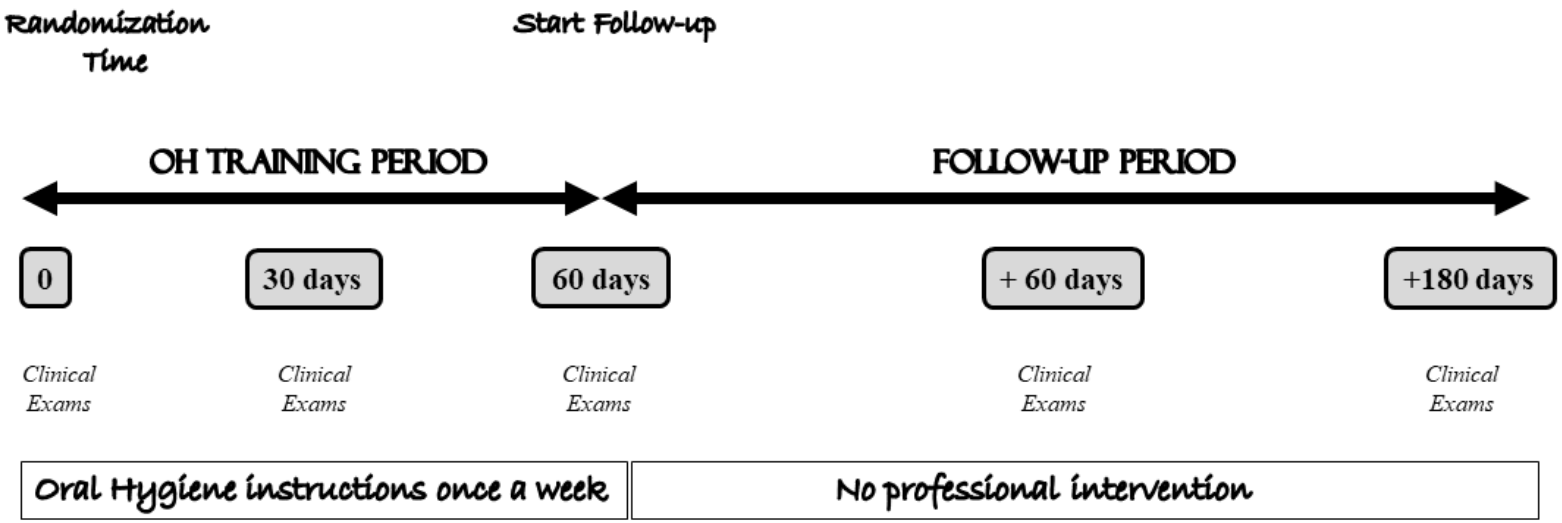
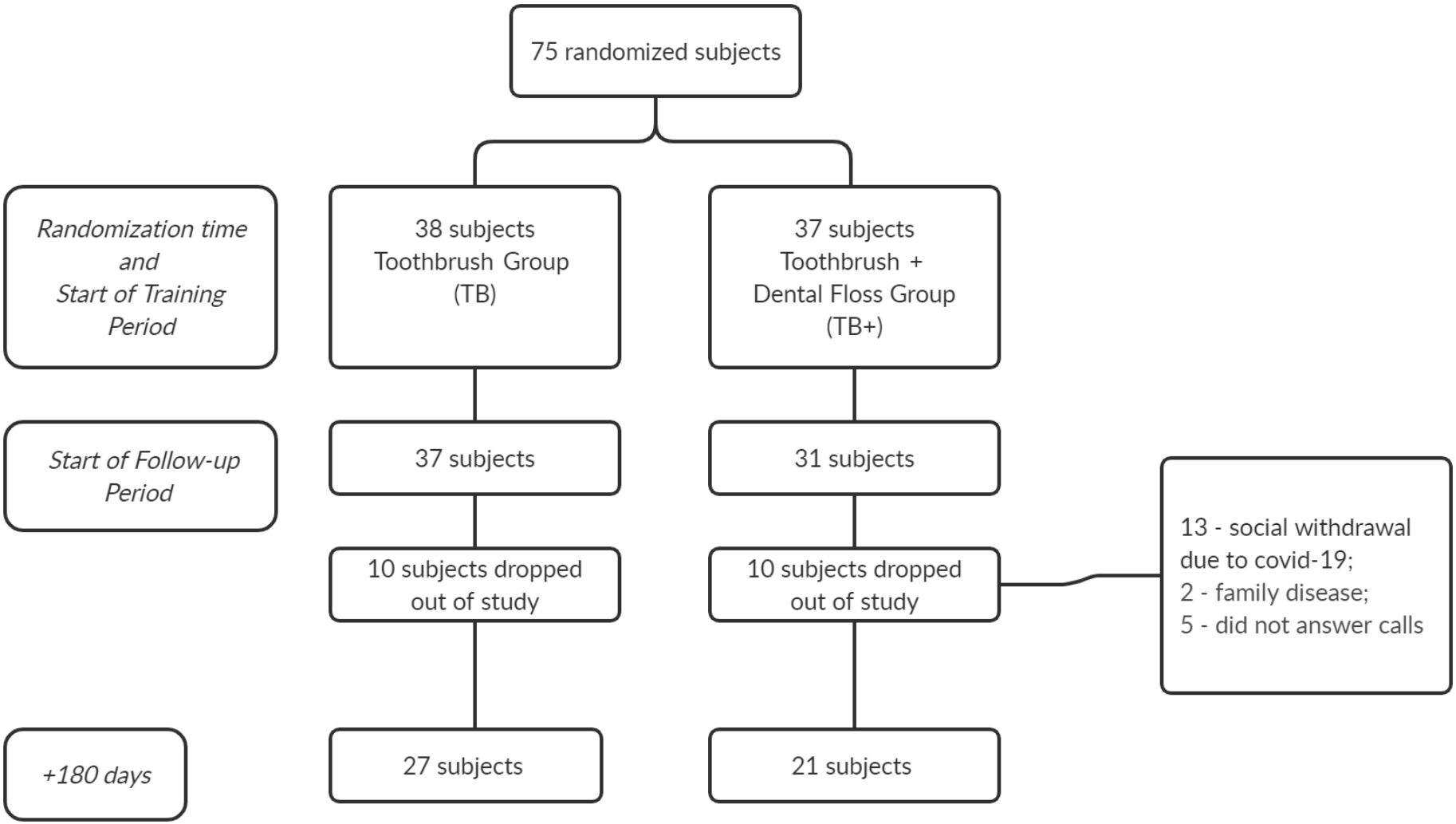


Figure 2 – Flowchart



Appendix A –

Mean percentage of sites with interproximal gingival bleeding (GI=2) (standard error) and interproximal Gingival Index mean (standard error) during the experimental period, for each experimental group, for analysis by intention to treat.

		TB	**TB+**
GI=2	*Follow up beginning*	22.33(2.27)^{Aa}	15.59(2.49)^{Ba}
	60 days	17.67(1.47)^{Ab}	15.69(1.60)^{Aa}
	180 days	18.12(1.63)^{Aab}	13.22(1.78)^{Ba}
GI	*Follow up beginning*	1.15(0.03)^{Cc}	1.10(0.04)^{Cc}
	60 days	1.14(0.03)^{Cc}	1.12(0.03)^{Cc}
	180 days	1.04(0.05)^{Cd}	0.95(0.05)^{Cd}

TB: toothbrushing group; TB+: toothbrushing supplemented by dental floss group.
Number of subjects at follow up beginning: 37 and 31 for TB and TB/F, respectively.
Number of subjects at 60 and 180 days: 27 and 21 for TB and TB/F, respectively.
Mixed linear models' analysis
Different uppercase letters (A, B) demonstrate intergroup differences regarding GI=2 ($P<0.05$)
Different lowercase letters (a, b) show intragroup differences regarding GI=2 ($P<0.05$)

ARTIGO 2– CORRELATION BETWEEN DENTAL PLAQUE ACCUMULATION AND GINGIVAL HEALTH IN PERIODONTAL MAINTENANCE PATIENTS USING SHORT OR EXTENDED PERSONAL ORAL HYGIENE INTERVALS

Este artigo foi submetido e aceito ao periódico *Journal of Clinical Periodontology* , ISSN: 1600-051X, Fator de impacto: 5.241, Qualis A1. As normas para publicação estão escritas no Anexo A.

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Journal of Clinical Periodontology, v. 48, n. 6, p. 834-842, Jun. 2021 (doi: 10.1111/jcpe.13448).

CORRELATION BETWEEN DENTAL PLAQUE ACCUMULATION AND GINGIVAL HEALTH IN PERIODONTAL MAINTENANCE PATIENTS USING SHORT OR EXTENDED PERSONAL ORAL HYGIENE INTERVALS

Running Title: Personal oral hygiene measures

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FUNDING INFORMATION: This study was partially supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

CONFLICT OF INTEREST: The authors declare that they have no conflicts of interest related to this study.

CLINICAL TRIALS: ClinicalTrials.gov: NCT02684682

ABSTRACT

Aims: To evaluate the correlation between bacterial dental plaque accumulation and gingival health in subjects with history of periodontitis attending a maintenance program including personal oral hygiene measures (pOH) at short and extended intervals. This study is a secondary analysis of a randomized-clinical-trial.

*Materials and Methods***:** Forty-two subjects were randomized into groups performing pOH at 12-, 24- or 48-hour intervals. The Plaque Index (PlI), Gingival Index (GI) and bleeding on probing (BoP) were recorded at baseline, 30 and 90 days. For the analysis, pOH groups were collapsed into subjects performing pOH at daily (G12/24) or extended (G48) intervals. Summary statistics and Spearman-correlations between plaque accumulation and gingival inflammation are presented.

Results: G12/24 and G48 subjects showed significant increases in plaque scores and percentage sites with gingival inflammation over the course of study. At 90 days, G48 subjects showed significantly greater GI and BoP scores than G12/24 subjects. Whilst PlI/GI correlations were not affected by pOH-interval, PlI/BoP correlations remained unchanged with short to increase with extended pOH-intervals.

Conclusion: pOH interval influences the correlation between bacterial dental plaque accumulation and gingival inflammation. Subjects using extended pOH-intervals exhibit an increased correlation allowing accumulation of bacterial dental plaque to the detriment of gingival health. (ClinicalTrials.gov: 50208115.9.0000.5346).

Keywords: dental plaque, gingivitis, oral hygiene, periodontitis

CLINICAL RELEVANCE:

Scientific rationale for study: Understanding correlations between plaque and gingival inflammation according personal oral hygiene (pOH) frequency in periodontal patients is important considering

assessment of plaque and gingival status are routine to establish needs for behavior modification and to indicate directions of professional interventions.

Principal findings: Subjects performing pOH extended intervals allow sufficient exposure to bacterial plaque to compromise gingival health.

Practical implications: Subjects undergoing periodontal maintenance need to perform efficient pOH every 12/24h to maintain plaque levels and gingival status compatible with health.

INTRODUCTION

Classic studies carried out in the 1960s and 1970s established the relationship between bacterial accumulations on teeth and initiation/reversion of gingivitis (Lang, Cumming, & Löe, 1973; Loe, Theilade, & Jensen, 1965). Studies using experimental models in dogs introduced the concept that gingivitis, if untreated, could progress into periodontitis (Lindhe, Hamp, & Loe, 1975). Subsequent evidence showed that among populations without essential oral hygiene traditions or professional dental care hosting substantial bacterial accumulations and calculus on their teeth, most subjects presented with mild to moderate periodontitis, a subpopulation even presenting with advanced periodontitis (Bælum, Fejerskov, & Karring, 1986; Loe, Anerud, Boysen, & Morrison, 1986). Taken together, accumulated evidence ascertains bacterial accumulation on the teeth a decisive prerequisite in the initiation/development of gingivitis and progression into periodontitis, gingivitis of particular significance since it is considered the precursor of periodontitis. Longitudinal studies support the observation that the development and progression of periodontitis is associated with increased presence of gingivitis (Ramseier et al., 2017; Schätzle et al., 2003). Conversely, in populations with a history of periodontitis no or minimal progression of periodontitis is consistent with absence of gingivitis (Axelsson, Nyström, & Lindhe, 2004; Lang, Adler, Joss, & Nyman, 1990).

To prevent/arrest gingivitis/periodontitis, and dental caries, it becomes essential to establish lifelong effective personal oral hygiene measures (pOH) achieved through a balance of oral hygiene events and their temporal recurrence (Axelsson, Nyström, & Lindhe, 2004), The American Dental

Association recommends that pOH should be performed twice daily (American Dental Association, 2019). Although these recommendations have become convention, there is limited evidence in support relative to gingival health (Kelner, Wohl, Deasy, & Formicola, 1974; Lang, Cumming & Löe, 1973). We have shown that in subjects with or without a history of periodontitis, pOH performed once/twice daily appears adequate to maintain gingival health (Pinto, De freitas, Dutra, Kantorski & Moreira, 2013, De Freitas et al., 2016; Maier et al., 2020).

Similar exposure to bacterial dental plaque has been shown to diverge in clinical expressions of gingivitis in a non-periodontitis population, suggesting distinct subpopulations defined as "high" and "low" responders (Trombelli et al., 2004). We have shown, also in a non-periodontitis population, that effective pOH performed daily decreased the correlation between dental plaque accumulation and gingival inflammation, while extended pOH-intervals allowed qualitative and quantitative changes of dental plaque that may favor establishment of bacterial species promoting gingival inflammation (de David et al., 2018). No report has elaborated correlations between dental plaque and gingival inflammation relative to pOH in a patient population participating in a periodontal maintenance program. Understanding of this correlation is important considering assessment of dental plaque and gingival health are routine in clinical practice to establish needs for patient behavior modification and to indicate directions of professional interventions, maybe in particular in periodontitis patients in a maintenance program. Although dental plaque is the main etiologic factor to gingival inflammation, dental plaque and gingival health may not be directly correlated. Factors, such as pOH frequency, the nature of the recorded indices may not be sensitive to determine minor changes in the gingival status or detect qualitative changes in dental plaque. In addition, individuals profiles linked to the immune response may influence the magnitude and direction of the correlation between dental plaque accumulation and gingival health. Our aim was to observe how closely these two variables are related. The present study is a secondary analysis of a randomized-clinical-trial with the aim to evaluate the correlation between bacterial dental plaque accumulation and gingival health in subjects with history of periodontitis attending a maintenance program including pOH at short and extended intervals. Considering that a weaker correlation between dental plaque accumulation and gingival inflammation

was observed in individuals without a history of periodontitis, performing pOH at short compared to extended intervals (de David et al., 2018), we hypothesize that similar correlations may be observed, considering individuals with a history of periodontitis.

MATERIALS AND METHODS

Study design

This study represents a secondary analysis of a randomized-clinical-trial performed November 2015 through February 2018 (Maier et al., 2020) at Federal University of Santa Maria (UFSM), Post-Graduate Program in Oral-Science, Dental School, RS, Brazil, methodology herein described in brief.

Sample

Periodontitis patients presenting with proximal attachment loss ≥3 mm at ≥2 non-adjacent teeth (Tonetti & Claffey, 2005), treated at the Post-Graduate Clinic, and attending a maintenance program using 4-6 month recalls, were eligible. Subjects should be 35 years or older, have at least 12 teeth, and exhibit limited gingival inflammation (≤7.5% of sites with Gingival Index score 2 and ≤25% of sites with bleeding on probing). Smokers, pregnant women, diabetics, subjects presenting with xerostomia, psychomotor disorders, fixed orthodontic appliances, subjects requiring antimicrobial prophylaxis, using drugs associated with gingival enlargement or having used antibiotic/anti-inflammatory drugs within 3 months of initiation of study were not included. Study entrance criteria were reviewed each examination during the experimental period.

Ethical considerations

Eligible subjects provided informed consent. This study was conducted following Guidelines and Norms Regulating Research involving humans. The research protocol was approved by the Ethics Committee in Research (CAAE: 50208115.9.0000.5346) and ClinicalTrials.gov (50208115.9.0000.5346).

Randomization and experimental period

At baseline, study subjects received coronal polishing and were randomized to conduct pOH at 12-, 24-, or 48-hour intervals. Randomization maintained confidentiality using opaque envelopes was generated using a computer program (Random Allocation Software, version 2.0). All subjects received a soft multi-bristle toothbrush (Colgate® Twister® Compact Head, New York, NY, USA), dental floss (Colgate®, New York, NY, USA) and/or interdental brushes (Bitufo®, São Paulo, SP, Brazil), fluoride dentifrice (Colgate® Anticaries Protection, 90g, New York, NY, USA), and a fluoride mouthwash (NovaDerme, 1500 ml, Santa Maria, RS, Brazil). They were instructed to perform pOH adhering to the study protocol without sharing dentifrice, mouthwash and dental floss with family members. To support compliance, they received a leaflet containing scheduled days they should perform pOH and contact information of an investigator to allow queries that may emerge. The subjects were instructed to use the mouthwash twice daily to provide comfort as a measure to support compliance. They were instructed to apply dentifrice at a single point across the brush to standardize dentifrice consumption (approximately 0.5g). At the end of study, the dentifrice tubes were weighed (Digital Balance Scale Professional-Mini, model-1480, Tanita Corporation, Tokyo, Japan) as a measure to assess study compliance. Questions regarding pOH frequency and possible reasons for non-compliance were addressed at this time.

Early stop guideline

Subjects who showed 30% or more sites with gingival bleeding during the experimental period were removed from study. They performed regular pOH and were reexamined weekly until restoring their baseline gingival status.

Clinical evaluation

Plaque index (PlI) (Silness & Loe, 1964), Gingival Index (GI) ((Löe & Silness, 1963) modified by (Löe, 1967)), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BoP) were

recorded at six-sites per tooth excluding third molars at initiation of study/baseline, and at 30 and 90 days using a periodontal probe (CP 15 UNC, Neumar, Brazil). Gingival inflammation (GI score 2) was recorded gently tracing the probe immediately inside the gingival margin at a 45º angle provoking bleeding. BoP was recorded up to 15 sec following PD recordings classified as absent or present.

Clinical examinations were performed immediately prior to scheduled pOH events. Thus, "true" PlI scores for each pOH-interval were recorded. First, PlI was evaluated by examiner APPR. Then patients performed pOH and examiner JM evaluated GI, PD, CAL, and BoP. Examinations were performed by two masked calibrated examiners. Training and calibration procedures were performed before initiation of the study using patients not included in the sample. Examiners were trained by an experienced examiner (CHCM) to assess PlI and GI. Examiner JM was evaluated before initiation of the study for PD (weighted kappa=0.98) and CAL (weighted kappa=0.96) reproducibility. Intra-examiner reproducibility was evaluated in one thousand sites using duplicate exams at a 1-hour interval.

Outcomes

The primary outcome of this study was to estimate the correlation between dental plaque accumulation and gingival health. To evaluate this correlation, we considered the PlI mean and two parameters to describe gingival inflammation: GI and BoP mean. To understand the correlation findings, we evaluated the behavior PlI, GI and BoP mean, besides fluctuations in PlI and GI scores between groups throughout study. Our secondary outcome was the correlation between gingival bleeding (GI score 2) and BoP.

Statistical analysis

In our previous study (Maier et al., 2020), pOH-intervals of 12 and 24 hours demonstrated the same pattern of dental plaque accumulation and gingival inflammation. Thus, in the present study, we collapsed them into one group (G12/24) to compare with the group that performed pOH every 48 hours (G48).

Summary statistics included means and standard deviations. Normal distribution was analyzed using the Kolmogorov-Smirnov-test. Intragroup and intergroup differences were determined using Linear Mixed Models. Spearman correlation coefficients between PlI and GI, and between PlI and BoP were calculated using the site as the unit of analysis. The Power to detect correlations at least of 0.20 was ever higher than 0.80. Besides, we calculated the correlation between GI score 2 and BoP categorizing the sites according PD at baseline (≤3mm and >3mm). Correlations between groups were evaluated using a general linear model after transforming for Z-values. Data were analyzed using a statistical software (SPSS, version 21.0, SPSS Inc., Chicago, IL, USA). The significance level was set at 5%.

RESULTS

Table 1 presents baseline patient demographics and clinical characteristics. No significant differences among groups were observed. The study subjects showed roughly 90% of the sites with a PD≤3mm and a mean CAL approximating 3 mm. One patient (48h group) reached the stop rule day 30, data from this examination was repeated day 90.

Table 2 shows PlI and GI behavior within experimental groups. The G12/24 group showed a significant increase for PlI and GI from baseline to 30 days without significant further change, while the G48 group also showed a significant increase for PlI and GI between baseline and day 30, it displayed additional increase at 90 days.

Figure 1 shows changes of PII and GI throughout the study. For both groups, G12/24 and G48, there was a reduction in healthy sites (GI score 0). Most of these sites migrated to GI score 1 showing visual inflammatory changes including edema and erythema at 90 days. In the G12/24 group, among the 13% sites that were healthy at baseline, 8% developed visual changes (GI score 1) and 5% gingival bleeding (GI score 2) by 90 days. In the G48 group, among the 39% sites that showed gingival health at baseline, 28% and 12%, respectively, developed visual changes and gingival bleeding.

When plaque scores were analyzed, the G12/24 group showed an approximately 20% plaque free site reduction over 90 days, 14% showed plaque detectable with periodontal probe (PlI score 1) and 9% showed visible plaque (PlI score 2) at 90 days. In G48 group, the plaque free site reduction was 50% from baseline to 90 days. The majority of sites (34%) showed visible plaque (PlI score 2) by 90 days.

Table 3 shows the percentage sites exhibiting gingival bleeding (GI score 2) and BoP. Although the G12/24 group showed a significant increase in the gingival bleeding over the 90 days of study, the average percentage sites did not exceed 10%. The G48 group completed the study with approximately 19% sites exhibiting gingival bleeding, a mean percentage statistically larger compared with the G12/24 group.

Both groups displayed BoP scores around 13% at baseline to significantly increase over the progress of study. Mean percentage BoP was statistically larger for the G48 group at 30 and 90 days (22% and 25%) compared with the G12/24 group (16% and 19%). Performing the analysis stratifying PD, the same behavior was observed for shallow sites PD≤3mm (Table 4). For sites with PD>3mm, the G12/24 group entered (46%) and exited (47%) the study with a similar mean percentage BoP sites, while the G48 group showed increases over time (from 37% at baseline to 45% at 90 days) although not statistically significant.

Table 5 details the study primary outcome, positive, statistically significant correlations between PlI and GI, and between PlI and BoP for both the G12/24 and G48 groups. The correlation between PlI and GI increased from baseline to 30 days for both groups while remained unchanged from 30 to 90 days. The correlation coefficient between PlI and BoP remained unchanged throughout the study for the G12/24 group while increasing over the study interval for the G48 group. Statistical differences were observed between correlation coefficients relative to short and extended pOH intervals. The correlation behavior between plaque and BoP was the same when the sites were categorized according interproximal and buccal/palatal surfaces (Appendix 1).

Examining the correlation between gingival bleeding (GI score 2) and BoP, positive statistically significant correlations increasing throughout the study were verified independent of group or PD.

Nevertheless, the G48 group produced larger correlation coefficients compared with the G12/24 group (Table 6).

DISCUSSION

The present study evaluated the correlation between bacterial dental plaque accumulation and gingival health in subjects with a history of periodontitis attending a maintenance program including pOH at 12-, 24-, or 48-hour intervals. Positive, statistically significant correlations were observed for all pOH intervals throughout the study. The correlations between PlI and GI showed the same behavior independent of pOH-interval. However, while correlation coefficient between PlI and BoP remained stable over time for the short/daily pOH interval group, the correlation coefficient increased throughout the 90-day observation interval for the extended/48h pOH group.

Deviating behavior between PlI/GI and PlI/BoP correlations according to pOH-interval may be explained by the nature of the indices, the GI based on a scoring system, while BoP represents a dichotomous index. When we calculated the correlation between PlI and gingival bleeding (GI score of 2), the behavior was the same as that observed for the PlI/BoP correlation, i.e., the correlation was maintained over time for the short/daily pOH-interval group while the correlation coefficient increased for the extended/48h pOH-interval group (Appendix 2).

We observed low PlI/GI and PlI/BoP correlation coefficients at baseline. Despite approximately 80% of the sites were plaque free at baseline, only 45% were free from gingival inflammation, about 50% of the sites presented with visual alterations (GI score 1) and 5% with gingival bleeding (GI score 2), independent of group. Discrepancy between absence of dental plaque and gingival inflammation may explain the low correlation coefficient values at baseline. We further verified that out of about 13.5% BoP positive sites at baseline, approximately 4% showed plaque (PlI score 1 or 2) resulting in low correlation value. It appears evident that study subjects carefully conducted pOH before the first appointment (baseline) producing low plaque scores. From thereon, throughout study, clinical

examinations were performed immediately prior to scheduled pOH events, securing “true” PlI scores for each pOH-interval.

Evaluating the correlation between PlI and GI scores, the correlation coefficients increased from baseline to 30 days without further verifiable change independent of pOH-interval. Throughout the study, there was an increase in sites with plaque (PlI score 1 or 2) with concomitant increases in gingival inflammation (GI score 1 or 2) resulting in increase of the correlation coefficient for both groups, mean GI changes mirroring PlI changes. For individuals performing pOH daily, the increase in PlI scores occurred over the first 30 days to remain unaltered through the end of study. The same pattern was verified for the GI. The G48 group displayed an increase in PlI and GI means from baseline to 30 days, to display another incremental increase for both parameters from 30 and 90 days. As consequence, the correlation coefficient for both groups, G12/24 and G48, increased from baseline to 30 days, thereafter no further change was verified.

Although the correlation coefficient values increased throughout the study, they remained weak. Lack of or weak correlation between PlI and clinical parameters to evaluate gingival inflammation were also observed in previous studies (Danielsen, Manji, Nagelkerke, Fejerskov & Baelum, 1989; Lie, Timmerman, van der Velden & van der Weijden, 1998). We consider some possible explanations for this result: (i) presence of other factors such as qualitative changes on dental biofilm and personal profile on immune inflammatory response, which could affect the finding correlations; (ii) and the use of a subjectively scored index (GI) introducing variability which might be of an issue when compared to objective measures of inflammation such as gingival crevicular fluid volume (GCF). In this way, Trombelli et al., (2004) verified a higher strength of correlation between GCF and dental plaque when compared to the correlation value between gingival bleeding and dental plaque.

In subjects without history of periodontitis, correlations between PII and GI were different. Correlations lowered over 30 days with short/daily while remained unchanged following extended pOH-intervals (every 48 or 72h)(de David et al., 2018). Inflammatory profile may be responsible for these differences, subjects with a history of periodontitis muster a hyperinflammatory response (Chapple et al., 2015; Trombelli et al., 2004).

The present study showed that the correlation coefficient between PlI and BoP remained stable over time for the short/daily pOH interval group while increasing throughout the 90 days of study for the extended/(every 48h) pOH-interval group. In the G12/24 group, the PlI mean virtually doubled from baseline to 30 days, to remain stable through 90 days. BoP scores increased throughout the study from 13.7% to 15.8% within 30 days, to reach 18.6% at 90 days, without nearing levels observed for the PlI scores, explaining the stability of the low correlation coefficient values for this group. In contrast, the G48 group showed a concomitant increase of PlI and BoP scores, i.e., for the extended pOH-interval a shorter exposition to the plaque appeared sufficient to promote clinical inflammation in the gingival tissues.

Designing the present study in 2015, the literature did not present a consensus regarding criteria defining a gingivitis case, subjects presenting with 15% of the gingival sites displaying inflammation were considered a localized gingivitis case. For the present study, we choose half this value (7.5%) GI score 2 positive sites to define "gingival health", an acceptable inflammatory status for subjects with history of periodontitis attending a maintenance program. In 2018, the new Classification of Periodontal and Peri-Implant Disease and Conditions indicated BoP a preferred parameter monitoring health and inflammation in gingival tissues (Lang & Bartold, 2018; Lang, Joss, & Tonetti, 1996) and selected a BoP score <10% to represent gingival health. Accordingly, our study groups, which presented with approximately 13% BoP positive sites at baseline, would represent successfully treated periodontitis patients with gingival inflammation. At the end of study, the G48 group showed a significantly greater mean percentage BoP positive sites compared with the G12/24 group (25.3% vs. 18.6%), confirming the extended pOH-interval allowed significantly increased plaque accumulation and gingival inflammation compared with the short interval.

The secondary outcome of the present study concerned the correlation between GI score 2/gingival bleeding and BoP. BoP is recorded as bleeding upon probing reaching tissue resistance at the "bottom" of a gingival sulcus or periodontal pocket. In absence of pocketing, it should be understood as bleeding provoked in the marginal gingiva. GI score 2/gingival bleeding in turn is recorded gently tracing the periodontal probe immediately ("1-2 mm") inside the gingival margin at a 45º angle

provoking bleeding (Lang & Bartold, 2018; Trombelli, Farina, Silva, & Tatakis, 2018). Thus, in sites with deeper PDs, GI score 2 indicates inflammatory alterations in the marginal gingiva, while BoP denotes deeper inflammatory changes. Previous observations indicate that the concordance between BoP and GI bleeding is dependent on the PD (Chaves et al., 1993). In the current sample, 90% of sites represented PDs≤3mm explaining a positive significant correlation between GI score 2 and BoP confirmed in the results. Similar correlations were explored for deeper sites (PD>3mm), however, only 10% of the sites represented a PD>3mm, only 3% a PD>4mm. This low representation of deeper sites may have biased correlation estimates thus interpreted with caution.

The present study revealed an increase in the correlation between GI score 2 (gingival bleeding) and BoP for both study groups. At baseline, a majority of BoP sites did not produce GI score 2 resulting in low correlation coefficients. This may be explained by that BoP positive sites were concentrated among deeper gingival sites reflecting inflammation in the depth of the sites without discernable inflammatory alterations within the immediate marginal gingiva detectable by GI bleeding. Throughout study, the increase in plaque scores promoting gingival inflammation increased the correlation between GI score 2 and BoP.

The current study represents a secondary analysis of a randomized clinical trial (Maier et al., 2020), which provides an assessment with a low risk of bias (Ahn & Ahn, 2010) configuring strength. The study sample includes subjects in systemic health (non-smokers) with a history of periodontitis attending a maintenance program presenting with ≤7.5% of the gingival sites with bleeding. These inclusion criteria reduce the external validity of our estimates considering: (i) the low inflammatory status which mirrors adequate pOH; and (ii) absence of known risk factors. Taken in account known effects of smoking (Bergström, 1990; Farina, Tomasi, & Trombelli, 2013; Preber & Bergström, 1985) and diabetes (Graves, Ding, & Yang, 2020; Salvi, Kandylaki, Troendle, Persson, & Lang, 2005) on gingival inflammation, it's possible that our correlations between dental plaque accumulation and gingival inflammation have been overestimated. Studies directly evaluating these questions are needed.

Summarizing, in subjects with a history of periodontitis attending a maintenance program the pOH-interval affects the correlation between bacterial dental plaque accumulation and gingival

inflammation, especially when characterized using BoP. Extended pOH-intervals increase while daily intervals maintain the correlation suggesting that subjects who choose to perform pOH at extended intervals allow sufficient exposure to bacterial plaque to compromise gingival health.

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Table 1. Mean (standard deviation) of the demographics and clinical characteristics at baseline according the groups

	G12/24 **n=28**	**G48** **n=14**	**P value**
Age (years)†	56.4 (7.4)	59.9 (8.1)	0.16
Gender n (%)‡			0.62
Male	12 (42.9)	6 (42.9)	
Female	16 (57.1)	8 (57.1)	
Periodontal Parameters			
PD (mm)†	2.34 (0.23)	2.35 (0.33)	0.96
BoP (%)†	13.77 (3.95)	13.06 (4.27)	0.60
CAL (mm) †	3.03 (0.81)	3.45 (1.20)	0.19
Gingival bleeding (GI 2)†	4.6 (2.0)	5.5 (2.3)	0.23
GI †	0.64 (0.26)	0.60 (0.21)	0.62
PlI †	0.22 (0.14)	0.28 (0.23)	0.38
PD≤3 (mm) n (%)‡	3314 (91%)	1480 (90.4%)	0.47
PD>3 (mm) n (%)‡	329 (9%)	158 (9.6%)	
Teeth number †	21.7 (3.9)	19.5 (4.5)	0.11

Abbreviations: PD: probing depth; BoP: bleeding on probing; CAL: clinical attachment level; GI: Gingival Index (Löe & Silness 1963, modified by Löe 1967); PlI: Plaque Index (Silness & Löe, 1964)
†T test
‡ Chi-square statistics

Table 2. Mean (SD) of Plaque Index (PlI) and Gingival Index (GI) according to experimental groups at baseline, 30 and 90 days

	PlI			GI		
	Baseline	**30d**	**90d**	**Baseline**	**30d**	**90d**
G12/24	0.22 (0.14) ^{A,a}	0.42 (0.24) ^{A,b}	0.49 (0.30) ^{A,b}	0.64 (0.26)^{A,a}	0.76 (0.22)^{A,b}	0.81 (0.25)^{A,b}
G48	0.28 (0.23) ^{A,a}	0.70 (0.42) ^{B,b}	1.10 (0.46) ^{B,c}	0.60 (0.21)^{A,a}	0.99 (0.18)^{B,b}	1.12 (0.13)^{B,c}

Linear Mixed Models
Different uppercase letters demonstrate intergroup differences (P<0.05)
Different lowercase letters show intragroup differences (P<0.05)
G12/24: n=28 at baseline and 30 d, n=26 at 90 d.
G48: n=14 at baseline and 30 d, n=12 at 90 d.

Table 3. Percentage mean (SD) of sites with gingival bleeding (GI 2) and bleeding on probing (BoP) according to experimental groups at baseline, 30 and 90 days

	Gingival bleeding (GI 2)			BoP		
	Baseline	**30d**	**90d**	**Baseline**	**30d**	**90d**
G12/24	4.6 (2.0)^{A,a}	7.0 (4.5)^{A,b}	9.4 (5.8)^{A,c}	13.7 (3.9)^{A,a}	15.8 (5.7)^{A,a}	18.6 (7.8)^{A,b}
G48	5.5 (2.3)^{A,a}	15.7 (10.1)^{B,b}	18.8 (10.3)^{B,c}	13.0 (4.2)^{A,a}	22.1 (9.8)^{B,b}	25.3 (10.9)^{B,b}

Linear Mixed Models
Different uppercase letters demonstrate intergroup differences (P<0.05)
Different lowercase letters show intragroup differences (P<0.05)
G12/24: n=28 at baseline; 30d and 90d n=26
G48: n=14 at baseline; 30d and 90d n=12

Table 4. Percentage mean (SD) of sites with bleeding on probing (BoP) according to initial probing depth (PD) throughout study

	PD≤3mm			PD>3mm		
	Baseline	**30d**	**90d**	**Baseline**	**30d**	**90d**
G12/24	10.7 (3.9)^{A,a}	13.2 (5.7)^{A,a}	16.0 (8.3)^{A,b}	45.7 (19.5)^{A,a}	40.1 (20.4)^{A,a}	46.5 (21.5)^{A,a}
G48	10.9 (3.5)^{A,a}	19.8 (9.7)^{B,b}	23.2 (10.7)^{B,b}	36.6 (23.0)^{A,a}	44.6 (23.9)^{A,a}	45.2 (22.5)^{A,a}

Linear Mixed Models
Different uppercase letters demonstrate intergroup differences (P<0.05)
Different lowercase letters show intragroup differences (P<0.05)
G12/24: n=28 at baseline; 30d and 90d n=26
G48: n=14 at baseline; 30d and 90d n=12

Table 5. Correlation between Plaque Index (PlI) and Gingival Index (GI), and between PlI and bleeding on probing (BoP) according to individual group at baseline, 30 and 90 days

	PlI/GI			**PlI/BoP**		
	Baseline	**30d**	**90d**	**Baseline#**	**30d#**	**90d#**
G12/24	0.16*	0.22*	0.23*	0.13*	0.11*	0.12*
G48	0.15*	0.26*	0.21*	0.08*	0.19*	0.22*

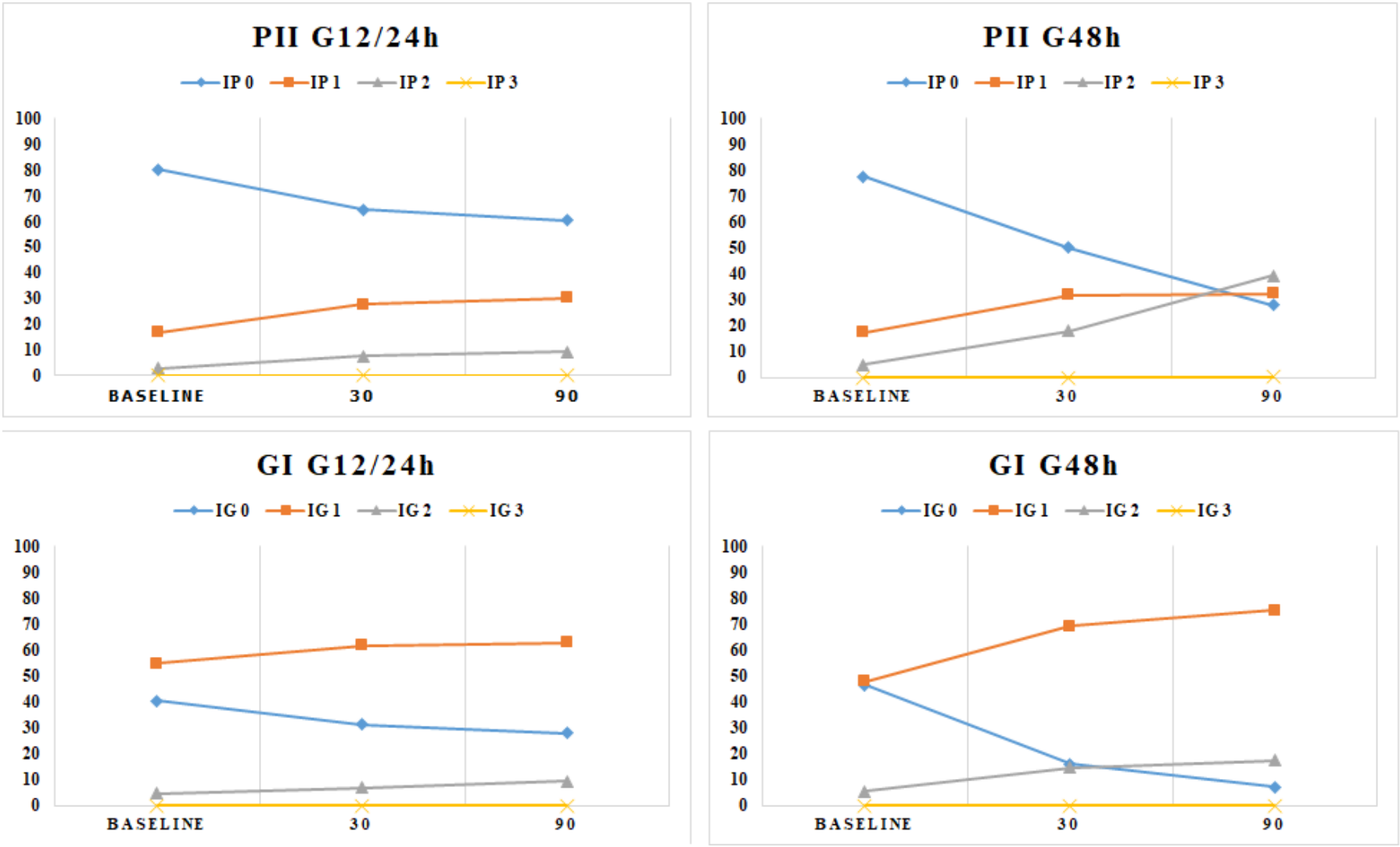
*Spearman correlation coefficient (P<0.001)
Represent statistical difference between groups in every experimental time (P<0.05)
G12/24: n=3642 at baseline; 30d and 90d n=3390
G48: n=1638 at baseline; 30d and 90d n=1380

Table 6. Correlation between Gingival Index (GI) score of 2 and bleeding on probing (BoP) according to individual group at baseline, 30 and 90 days

	All sites			**PD≤3mm**			**PD>3mm**		
	Baseline	**30d**	**90d**	**Baseline**	**30d**	**90d**	**Baseline**	**30d**	**90d**
G12/24	0.17*	0.23*	0.28*	0.17*	0.25*	0.28*	0.13*	0.16*	0.27*
G48	0.19*	0.30*	0.35*	0.17*	0.32*	0.35*	0.28*	0.17*	0.33*

*Spearman correlation coefficient (P<0.001)
All sites: G12/24 n=3642 at baseline, 30d and 90d n=3390; G48 n=1638 at baseline, 30d and 90d n=1380.
PS≤3mm: G12/24 n=3313 at baseline, 30d and 90d n=3066; G48 n=1480 at baseline, 30d and 90d n=1228.
PS>3mm: G12/24 n=329 at baseline, 30d and 90d n=324; G48 n=158 at baseline, 30d and 90d n=152.

Figure 1 – Percentage of Plaque Index (PlI) and Gingival Index (GI) scores (0, 1, 2 and 3) for study groups using daily (G12/24h) or extend (G48h) pOH intervals.



Appendix 1:

Correlation between Plaque Index (PlI) and bleeding on probing (BoP) according to group at baseline, 30 and 90 days for interproximal surfaces

	PlI/BoP		
	Baseline	**30d**	**90d**
G12/24	0.12*	0.12*	0.12*
G48	0.06*	0.19*	0.18*

G12/24: n=2428 at baseline; 30d and 90d n=2260
G48: n=1092 at baseline; 30d and 90d n=920

Correlation between Plaque Index (PlI) and bleeding on probing (BoP), according to group at baseline, 30 and 90 days for buccal and lingual/palatal surfaces.

	PlI/BoP		
	Baseline	**30d**	**90d**
G12/24	0.14*	0.10*	0.09*
G48	0.11*	0.19*	0.29*

G12/24: n=1214 at baseline; 30d and 90d n=1130
G48: n=546 at baseline; 30d and 90d n=460

Appendix 2

Correlation between Plaque Index (PlI) and Gingival Index (GI) score of 2, according to individual group at baseline, 30 and 90 days

	PlI/GI=2		
	Baseline	**30d**	**90d**
G12/24	0.10*	0.12*	0.13*
G48	0.06*	0.19*	0.17*

*Spearman correlation coefficient (P<0.001)
G12/24: n=28 at baseline; 30d and 90d n=26
G48: n=14 at baseline; 30d and 90d n=12

DISCUSSÃO

A manutenção de hábitos de higiene bucal efetivos ao longo do tempo é um desafio na prática clínica diária. Os pacientes, muitas vezes, estão cientes sobre a necessidade de higiene bucal e realizam uma frequência adequada. Entretanto, a qualidade da escovação e remoção de placa interdental não são suficientes para atingir e ou manter a saúde gengival. O primeiro artigo da tese é a fase de acompanhamento de um estudo que incluiu indivíduos jovens, média de idade de 23 anos, e apresentavam gengivite generalizada (média de ISG=38%). A fase inicial do estudo compreendeu 8 sessões semanais de orientação e motivação de higiene de acordo com os grupos (Escova + Fio versus Escova). Após este período, observamos redução para ambos os grupos em torno de 50% nos escores de ISG que se mantiveram ao longo de 180 dias. Além disso, o grupo que utilizou o fio dental associado a escova apresentou maior redução de inflamação proximal quando comparado ao grupo que utilizou apenas escova. Estes resultados nos demonstram o quanto é importante o cirurgião-dentista dedicar tempo para orientar e motivar os pacientes para realização de uma higiene adequada. A desorganização de placa proximal com o uso do fio dental é uma prática que requer destreza e motivação do paciente. O indivíduo que consegue visualizar melhora nas condições gengivais, ausência de sangramento e desconforto, hálito agradável, compreende a importância da higiene com fio dental. O retorno a cada 6 meses é uma orientação comum nos consultórios odontológicos. Nossos resultados confirmam esta afirmação, pois nossos pacientes se mantiveram saudáveis durante os 180 dias de acompanhamento sem intervenção profissional.

Estudos anteriores do nosso grupo observaram que em indivíduos com hábitos de higiene adequados (ISG ≤ 7,5%), frequência diária (12/24h) de desorganização de placa é suficiente para manter saúde gengival tanto em pacientes sem perda de inserção prévia (PINTO et al., 2013) quanto em manutenção periodontal (MAIER et al., 2020). Pacientes costumam relatar frequência de escovação 3 vezes ao dia ou a cada alimentação. Isso acontece devido a crença popular, presente em muitas propagandas de dentifrício ou enxaguantes bucais, que devemos higienizar os dentes devido aos resíduos alimentares. O profissional de odontologia ao instruir seus pacientes quanto à higiene bucal, deve esclarecer que esse procedimento tem como objetivo principal a remoção do fator etiológico das doenças periodontais e cárie

dentária, o biofilme dentário. Essa desorganização meticulosa do biofilme precisa ser realizada diariamente, atingindo todas as faces dentárias e em especial a região interproximal, que exige dispositivos específicos determinados pelo profissional de acordo com a necessidade de cada paciente.

No segundo artigo da tese, observamos que o coeficiente de correlação entre índices de placa e inflamação gengival foi diferente nestes perfis de pacientes. Em indivíduos sem histórico de periodontite, a correlação entre IP (Índice de Placa) e IG (Índice Gengival) diminuiu ao longo dos 30 dias para o grupo de intervalos diários (12/24h), enquanto permaneceu inalterada no grupo de intervalo estendido (48/72h) (DE DAVID et al., 2018). Já no perfil de pacientes em manutenção periodontal, os coeficientes de correlação aumentaram do baseline aos 30 dias para ambos os grupos (intervalos diários/ estendidos) (REINIGER et al., 2021). Esta diferença de correlação nos dois perfis de pacientes pode ser explicada devido a diferença de suscetibilidade. Assim, enfatizamos a importância das consultas de manutenção periodontal. Os periodontistas devem estar atentos ao controle de placa destes pacientes e orienta-los sobre a importância de visitas periódicas. Indivíduos que já tiveram periodontite precisam estar cientes sobre a necessidade de manter os tecidos gengivais saudáveis, a fim de evitar nova progressão da doença.

CONCLUSÃO

Para prevenir/interromper a gengivite/periodontite e cáries dentárias, é essencial estabelecer medidas de higiene bucal eficazes ao longo da vida, alcançadas por meio de equilíbrio entre os eventos de higiene bucal e sua recorrência temporal (AXELSSON; NYSTRÖM; LINDHE, 2004).

Considerando este contexto, os resultados do primeiro artigo da tese mostram que treinamento semanal durante 8 semanas promove redução de inflamação gengival proximal e manutenção deste resultado durante 6 meses. Dentistas necessitam investir mais tempo treinando e motivando seus pacientes a obter hábitos adequados de autocontrole mecânico de placa, independente dos dispositivos indicados. Além disso, o uso do fio dental se mostrou essencial para redução de inflamação gengival em indivíduos que apresentam papila preenchendo espaço interdental.

O segundo artigo da tese esclarece que indivíduos com histórico de periodontite incluídos em manutenção periodontal, e que apresentavam desorganização de placa efetiva no início do estudo (IG2 baseline <7,5%), demonstraram aumento da correlação entre placa e inflamação gengival quando realizavam intervalos estendidos de higiene bucal (48h). No decorrer do tempo, os escores de placa aumentaram concomitante com o aumento da inflamação gengival. Por outro lado, indivíduos que realizaram higiene bucal com intervalos menores (diariamente), embora tivessem experimentado aumento da quantidade de placa, não apresentaram aumento da inflamação gengival, não alterando a correlação entre os dois parâmetros observada no baseline. Isso significa que em pacientes periodontais em manutenção, efetiva higiene bucal diária permite que a placa formada não atinja patogenicidade suficiente para resultar em disbiose.

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ANEXO A- NORMAS PARA PUBLICAÇÃO NO PERIÓDICO *JOURNAL OF CLINICAL PERIODONTOLOGY*

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The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1.GENERAL

Journal of Clinical Periodontology publishes original contributions of high scientific merit in the fields of periodontology and implant dentistry. Its scope encompasses the physiology and pathology of the periodontium, the tissue integration of dental implants, the biology and the modulation of periodontal and alveolar bone healing and regeneration, diagnosis, epidemiology, prevention and therapy of periodontal disease, the clinical aspects of tooth replacement with dental implants, and the comprehensive rehabilitation of the periodontal patient. Review articles by experts on new developments in basic and applied periodontal science and associated dental disciplines, advances in periodontal or implant techniques and procedures, and case reports which illustrate important new information are also welcome.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Journal of Clinical Periodontology*. Authors are encouraged to visit Wiley-Blackwell's Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

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Authors submitting a paper do so on the understanding that the manuscript have been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

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It is a requirement that all authors have been accredited as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under Acknowledgements. Please note that it is a requirement to include email addresses for all co-authors at submission. If any of the email-addresses supplied are incorrect the corresponding author will be contacted by the journal administrator.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited.

2.2.Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

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Clinical trials should be reported using the CONSORT guidelines available at. A should also be included in the submission material.

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- Conflict of Interest and Source of Funding
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For clinical trials, it is encouraged that the abstract finish with the clinical trial registration number on a free public database such as clinicaltrials.gov.

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These must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

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- Introduction,
- Materials and Methods,
- Results and Discussion.
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The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Please see Sample Manuscript.

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Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced. As a condition of publication, authors are required to make materials and methods used freely available to academic researchers for their own use. This includes antibodies and the constructs used to make transgenic animals, although not the animals themselves.
(a) Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist should also be included in the submission material. If your study is a randomized clinical trial, you will need to fill in all sections of the CONSORT Checklist. If your study is not a randomized trial, not all sections of the checklist might apply to your manuscript, in which case you simply fill in N/A.

Journal of Clinical Periodontology encourages authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following free, public clinical trials registries: www.clinicaltrials.gov, http://clinicaltrials.ifpma.org/clinicaltrials/. The clinical trial registration number and name of the trial register will then be published with the paper.
(b) Statistical Analysis: As papers frequently provide insufficient detail as to the performed statistical analyses, please describe with adequate detail. For clinical trials intention to treat analyses are encouraged (the reasons for choosing other types of analysis should be highlighted in the submission letter and clarified in the manuscript).

(c) DNA Sequences and Crystallographic Structure Determinations: Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly

(d) Experimental Subjects: Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.
Results: should present the observations with minimal reference to earlier literature or to possible interpretations.
Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The discussion section should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

The discussion may usefully be structured with the following points in mind (modified from the proposal by Richard Horton (2002), The Hidden Research Paper, The Journal of the American Medical Association, 287, 2775-2778). Not all points will apply to all studies and its use is optional, but we believe it will improve the discussion section to keep these points in mind.

Summary of key finding

* Primary outcome measure(s)

* Secondary outcome measure(s)

* Results as they relate to a prior hypothesis

Strengths and Limitations of the Study

* Study Question

* Study Design

* Data Collection

* Analysis

* Interpretation

* Possible effects of bias on outcomes

Interpretation and Implications in the Context of the Totality of Evidence

* Is there a systematic review to refer to?

* If not, could one be reasonably done here and now?

* What this study adds to the available evidence

* Effects on patient care and health policy

* Possible mechanisms

Controversies Raised by This Study Future Research Directions

* For this particular research collaboration

* Underlying mechanisms

* Clinical research

5.4. Clinical Innovation Reports

These are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards**.** The word limit for clinical innovation reports is 3000 words, and up to 12 items (figures and tables) may be included. Additional items can be included as supplementary files online (please see 5.9 below).

The main text of Clinical Innovation Reports should be organized with

- Introduction,
- Clinical Innovation Report,
- Discussion and Conclusion
- References (see section 5.7)

5.5. Case Reports

Case reports illustrating unusual and clinically relevant observations are acceptable but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

The main text of Case Reports should be organized with

- Introduction,
- Case report,
- Discussion and Conclusion
- References (see section 5.7)

5.6. Reviews

Reviews are selected for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should take a broad view of the field rather than merely summarizing the authors´ own previous work, so extensive citation of the authors´ own publications is discouraged. The use of state-of-the-art evidence-based systematic approaches is expected. Reviews are frequently commissioned by the editors and, as such, authors are encouraged to submit a proposal to the Journal. Review proposals should include a full-page summary of the proposed contents with key references.

The word limit for reviews is 4000 words.

The main text of Reviews should be organized with

- Introduction,
- Review of Current Literature,
- Discussion and Conclusion
- References (see section 5.7)

5.7. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

A sample of the most common entries in reference lists appears below. Please note that a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the APA FAQ. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Journal article

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. The American Journal of Psychiatry, 159, 483–486. doi:10.1176/appi.ajp.159.3.483

Book

Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

Chapter in an Edited Book

Borstrøm, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), Dyslexia: Biology, cognition and intervention (pp. 235–253). London: Whurr.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from *http://www.youtube.com/watch?v=Vja83KLQXZs*

Please note that all unpublished papers (submitted or in press) included in the reference list should be provided in a digital version at submission. The unpublished paper should be uploaded as a supplementary file for review.

5.8. Tables, Figures and Figure Legends

Tables: should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.
Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Each copy should be marked with the figure number and the corresponding author's name. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size. Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by thin spaces (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar

itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Guidelines for Cover Submission If you would like to send suggestions for artwork related to your manuscript to be considered to appear on the cover of the journal, please follow these guidelines.

Permissions: If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

Figure Legends: should be a separate section of the manuscript, and should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

5.9. Supplementary Material

Supplementary material, such as data sets or additional figures or tables that will not be published in the print edition of the Journal but which will be viewable in the online edition, can be uploaded as 'Supporting information for review and online publication only'.

Please see **http://authorservices.wiley.com/bauthor/suppmat.asp** for further information on the submission of Supplementary Materials.