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VANESSA RENATA SANTOS DA SILVA

TRATAMENTO PERIODONTAL EM INDIVÍDUOS DIABÉTICOS TIPO 2: AVALIAÇÃO CLÍNICA, GLICÊMICA E DE BIOMARCADORES

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A Comissão Julgadora dos trabalhos de Defesa de Tese de DOUTORADO, intitulada "TRATAMENTO PERIODONTAL EM INDIVÍDUOS DIABÉTICOS TIPO 2: AVALIAÇÃO CLÍNICA, GLICÊMICA E DE BIOMARCADORES" em sessão pública realizada em 22 de Janeiro de 2013 considerou a candidata Vanessa Renata Santos da Silva aprovada.

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RESUMO

Objetivos: Considerando as evidências de que o diabetes e a periodontite apresentam uma relação de influência mútua, os objetivos desse trabalho, composto por quatro estudos científicos, foram: *Estudo 1*- Avaliar os níveis do ligante do receptor do ativador do fator nuclear kappa-ß (RANKL) e osteoprotegerina (OPG) no fluido gengival (FG) de indivíduos com periodontite crônica (PC) portadores de diabetes tipo 2 bem controlados ou com controle glicêmico insatisfatório, antes,3 e 6 meses após terapia periodontal básica; *Estudo 2* - Avaliar os níveis do fator de necrose tumoral (TNF)-α, interferon (INF)-γ, interleucina (IL)-4, IL-17 e IL-23 no FG do mesmo perfil de indivíduos do *Estudo 1*, antes, 3 e 6 meses após terapia periodontal básica; *Estudo 3* - Avaliar, em 12 meses, os efeitos da raspagem e alisamento radicular (RAR) de boca-toda em curto prazo (FMSRP), comparativamente à RAR por quadrante, nos parâmetros clínicos e níveis de biomarcadores em diabéticos tipo 2 com PC; *Estudo 4* - Avaliar, em 12 meses, os efeitos clínicos e glicêmicos da desinfecção de boca-toda (FMD) em diabéticos tipo 2 com PC. **Material e Métodos:** 18 diabéticos com hemoglobina glicada (HbA1c) ≤ 8% e 20 com HbA1c > 8% foram tratados nos *Estudos 1* e *2*. Em ambos estudos, os níveis dos biomarcadores foram analisados por ELISA, antes e em 3 e 6 meses pós-terapia. No *Estudo 3*, 17 indivíduos receberam FMSRP e 17 indivíduos a RAR por quadrante. Os níveis de 7 biomarcadores (método ELISA) e os parâmetros clínicos foram avaliados antes, em 3, 6 e 12 meses pós-terapias. No *Estudo 4*, os pacientes receberam o protocolo de FMD (n=19) ou FMSRP e placebo (n =19). Parâmetros clínicos e os níveis de HbA1c foram avaliados antes, em 3, 6 e 12 meses pós-terapias. **Resultados**: No *Estudo 1*, diabéticos com controle glicêmico insatisfatório apresentaram níveis mais elevados de RANKL e RANKL/OPG em 3 e 6 meses pós-terapia ($p<0,05$). No *Estudo 2*, os níveis de IL-17 estavam aumentados nos indivíduos com controle insatisfatório e os níveis de INF-γ aumentados nos que apresentavam controle satisfatório, em todos os tempos ($p<0,05$). No *Estudo 3*, todos os parâmetros clínicos melhoraram após ambas terapias ($p<0,05$), sem diferenças entre os grupos terapêuticos ($p>0,05$). Em geral, não houve diferenças consideráveis para os níveis locais de biomarcadores entre os grupos de tratamentos ($p>0,05$). No *Estudo 4*, os parâmetros clínicos melhoraram pós-terapias ($p<0,05$), em todos os tempos, sem diferenças entre os grupos ($p>0,05$). Não foram observadas mudanças significativas nos níveis de HbA1c após ambos tratamentos ($p>0,05$). **Conclusões**: De acordo com o *Estudo 1*, os níveis de RANKL/OPG em sítios com periodontite tratados e não-tratados podem ser negativamente influenciados pelo controle glicêmico insatisfatório. O *Estudo 2* indicou uma predominância de citocinas próinflamatórias Th1 ou Th17 nos sítios com PC de acordo com o controle glicêmico. O *Estudo 3* demonstrou que FMSRP e RAR por quadrante não diferiram em benefícios clínicos e modulação de biomarcadores em 12 meses. De acordo com o *Estudo 4*, FMSRP mais placebo e FMD não diferiram em relação aos parâmetros clínicos e glicêmicos no tratamento de diabéticos tipo 2.

Palavras-chave: diabetes melito, periodontite crônica, citocinas, ligante RANK, osteoprotegerina, tratamento periodontal.

ABSTRACT

Aims: Considering the evidence that diabetes mellitus and periodontitis have a mutual relationship, the aim of this work, composed by four scientific studies, were: *Study 1:* To evaluate the levels of the receptor activator of NF-$_{K}$B ligand (RANKL) and osteoprotegerin (OPG) in gingival crevicular fluid (GCF) from poorly- and well-controlled type 2 diabetic subjects with chronic periodontitis (CP) at baseline, 3 and 6 months after basic periodontal therapy; *Study 2:* To evaluate the levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-4, IL-17 and IL-23 in the GCF of the same patient profile from the *Study 1*, at baseline, 3 and 6 months after basic periodontal therapy ; *Study 3:* To evaluate the effects of full-mouth (FM) scaling and root planing (SRP), compared to the quadrant SRP, on clinical parameters and biomarker levels in type 2 diabetic with CP up to 12 months; *Study 4:* To evaluate the clinical and glycemic effects of the full-mouth disinfection (FMD) protocol in type 2 diabetic with CP up to 12 months. **Material and methods:** 18 type 2 diabetic subjects presenting glycated hemoglobin (HbA1c) ≤ 8% and 20 presenting HbA1c > 8% were treated in the *Studies 1* and *2*. In both studies, the biomarker levels were analyzed by ELISA at baseline, 3 and 6 months post-therapy. In the *Study 3*, 17 subjects received FMSRP and 17 subjects received quadrant SRP. The levels of 7 biomarkers (ELISA method) and clinical parameters were evaluated before and at 3, 6 and 12 months post-therapies. In the *Study 4*, the patients received FMD protocol (n=19) or FMSRP plus placebo (=19). Clinical parameters and HbA1c levels were evaluated before and at 3, 6 and 12 months post-therapies. **Results**: In the *Study 1*, poorly-controlled diabetics presented higher levels of RANKL and RANKL/OPG at 3 and 6 months post-therapy ($p<0.05$). In the *Study 2*, the levels of IL-17 were higher in the poorly-controlled ($p<0.05$), whereas the levels of IFN-γ were increased in better-controlled subjects at all experimental groups ($p<0.05$). In the *Study 3*, clinical parameters improved after both therapies ($p<0.05$), without differences between treatment groups at any time-points ($p>0.05$). Overall, there were no considerable differences in the local levels of the biomarkers studied between treatment groups ($p>0.05$). In the *Study 4*, all clinical parameters improved after therapies ($p<0.05$), without differences between groups at any time-point ($p>0.05$). There were no significant changes in the HbA1c levels for both treatments ($p>0.05$). **Conclusions:** According to the *Study 1*, RANKL/OPG levels in untreated and treated periodontitis sites may be negatively influenced by a poor glycemic control. The *Study 2* indicated a predominance of pro-inflammatory Th1- or Th17-cytokines in sites of CP, according to the glycemic control. The *Study 3* demonstrated that FMSRP and quadrant SRP did not differ in relation to clinical benefits and biomarker modulation up to 12 months. According to the *Study 4*, FMSRP plus placebo and FMD did not differ in relation to clinical and glycemic parameters in the treatment of type 2 diabetics.

Key Words: diabetes mellitus, chronic periodontitis, cytokines, RANK ligand, osteoprotegerin, periodontal treatment.

LISTA DE ABREVIATURAS

DM	Diabetes Melito
OMS	Organização Mundial da Saúde
HbA1c	Hemoglobina glicada
PS	Profundidade de sondagem
SS	Sangramento à sondagem
TNF	Fator de necrose tumoral
PGE	Prostaglandina
IL	Interleucina
INF	Interferon
Th	Célula T auxiliar
T-reg	Células T regulatória
OPG	Osteoprotegerina
RANKL	Ligante do receptor do ativador do fator nuclear kappa-ß
RANK	Receptor do ativador do fator nuclear kappa-ß
PCR	Reação em cadeia da polimerase
ELISA	*Enzyme-linked immunosorbent assay* -Ensaio
RAR	Raspagem e alisamento radicular
TM	Terapia mínima
TF	Terapia frequente

SUMÁRIO

1. INTRODUÇÃO

1.1 Diabetes melito

Nos últimos anos, o diabetes melito (DM) tem emergido como uma doença com altos índices de incidência e prevalência no Brasil e no mundo. O DM se caracteriza como um grupo de desordens clínicas e genéticas heterogêneas, afetando o metabolismo de carboidratos, lipídios e proteínas, que resulta em hiperglicemia crônica (Organização Mundial da Saúde-OMS, 1999; American Diabetes Association-ADA, 2012). A hiperglicemia, por sua vez, é gerada pela resistência periférica à ação da insulina no tecido muscular, adiposo e no fígado e, a deficiência da produção deste hormônio pelas células ß pancreáticas, podendo ocorrer ainda a combinação de ambos os fatores (Cnop et al., 2005). A expressão do gene da insulina é restrita as células ß pancreáticas, o que confere a esse tipo celular o controle total sobre o único hormônio hipoglicemiante existente (Orci, 1985). Dentre as conseqüências causadas pelo DM em longo prazo estão danos, disfunções e/ou falhas de vários órgãos que podem levar ao desenvolvimento de complicações crônicas como retinopatia, neuropatia, nefropatia, microangiopatia e algumas manifestações bucais, dentre as quais se destaca a periodontite (Mealey; Ocampo, 2007).

A classificação etiológica do DM inclui o DM tipo 1, DM tipo 2 e o DM gestacional. Os diabetes tipos 1 e 2 eram denominados anteriormente como DM insulino-dependente e DM não insulino-dependente, respectivamente. Esta classificação foi revista pois, em grande parte dos casos, era complexo diferenciar os pacientes portadores de DM insulino-dependente dos pacientes com DM não insulino-dependente que acabavam necessitando de tratamento com insulina para controle da hiperglicemia em algum estágio da doença. A classificação atual, que se baseia na etiologia ao invés do tipo de tratamento, enfatiza a história e as características dos pacientes para determinar a etiologia e o tipo provável de DM (Cnop et al., 2005; ADA, 2012).

O DM tipo 1 é decorrente de um processo auto-imune que gera destruição das células ß pancreáticas, pela indução da apoptose celular, geralmente causando deficiência absoluta de insulina. Este processo, mediado pelo sistema imunológico, ocasiona um quadro permanente de hiperglicemia característico da patologia (Report of the..., 1997). O DM tipo

1 constitui uma pequena porcentagem dos casos de DM; enquanto o tipo 2 é responsável pela maioria dos casos. Geralmente acomete crianças e adolescentes, embora alguns estudos relatem que 15 a 30% dos casos ocorram em indivíduos com mais de 30 anos de idade (Laakso; Pyorala, 1985).

No desenvolvimento do DM tipo 2, ocorre a incapacidade das células ß pancreáticas em responder à crescente demanda periférica de insulina, observada durante a evolução progressiva da insulino-resistência em indivíduos intolerantes à glicose (Orci, 1985). A patogênese do DM tipo 2 é mais variável do que a do tipo 1, compreendendo diferentes graus de falhas das células ß pancreáticas, relativo a vários graus de resistência a insulina. Com a progressão dessa condição, a produção de insulina geralmente diminui e os pacientes têm uma deficiência de insulina relativa associada à resistência insulínica periférica (Rhodes, 2005). Este é o tipo mais comum de DM, geralmente acomete indivíduos mais velhos e pode ser regulado pela modificação da dieta e utilização de hipoglicemiantes orais, e em alguns casos, a utilização de insulina pode ser necessária (Garcia et al., 2001). O diagnóstico do DM é frequentemente descoberto em decorrência de resultados anormais de exames de sangue ou de urina realizados em avaliação laboratorial, e em alguns casos quando já há a manifestação de alguma complicação relacionada ao DM. Sendo assim, muitos indivíduos podem não ser diagnosticados por muitos anos porque a hiperglicemia aparece gradualmente e, geralmente só é detectada após a manifestação de algum sintoma associado a doença (Defronzo; Ferrannini, 1991).

No ano de 2000, a Organização Mundial da Saúde (OMS) estimava em 177 milhões o número de portadores de DM em todo o mundo. Em 2025, a previsão é de que essa população atinja quase o dobro: 350 milhões de diabéticos. O aumento da expectativa de vida da população, a urbanização crescente e a adoção de estilos de vida pouco saudáveis como sedentarismo, dieta inadequada e obesidade, são os grandes responsáveis pelo aumento da incidência e prevalência do DM. No Brasil, aproximadamente 6.2 milhões de pessoas acima de 18 anos tinham diabetes no ano de 2006, de acordo com a Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (Vigitel) do Ministério da Saúde. Rezende et al. (2010), em um estudo transversal hipotético, estimaram que existem cerca de 6.48 milhões de brasileiros com DM tipo 2. Mendes et al. (2010)

avaliaram em um estudo multicêntrico, 6671 brasileiros com DM. Os autores observaram que 979 (15%) eram portadores de DM tipo 1 e 5692 (85%) de DM tipo 2, sendo que, a prevalência de indivíduos com controle glicêmico inadequado [hemoglobina glicada (HbA1c) ≥7] foi de 90% em brasileiros com DM tipo 1 e 73% em brasileiros com DM tipo 2. Cheung et al. (2009) analisaram a prevalência de DM em 17.306 americanos entre 1999 e 2006. Os autores concluíram que a prevalência de DM aumentou em 6,5% de 1999 a 2002 e em 7,8% de 2003 a 2006.

1.2 Influência do DM na periodontite

Periodontite é uma doença infecciosa-inflamatória induzida por biofilme bacteriano específico, que leva a inflamação gengival, destruição dos tecidos periodontais e perda óssea alveolar (Socransky; Haffajje, 1992; Kinane; Mark Bartold, 2007). A respeito da etiologia, já está bem estabelecido que o biofilme bacteriano patogênico é o fator etiológico das periodontites. Entretanto, alguns fatores sistêmicos e hábitos (ex.: tabagismo, DM, estresse, osteoporose, obesidade, etc) podem exercer uma influência negativa no estabelecimento, progressão e gravidade das periodontites por meio de mecanismos imunoinflamatórios ainda não totalmente elucidados (Mercado et al., 2003; Choi et al., 2011). Várias evidências científicas demonstraram que o DM é um fator de risco para as doenças periodontais, influenciando negativamente o desenvolvimento das mesmas (Emrich et al., 1991; Löe, 1993; Grossi et al., 1994; Firatli et al., 1996). Por outro lado, as periodontites parecem também contribuir para a dificuldade do controle glicêmico em indivíduos diabéticos (Janket et al., 2005; Darré et al., 2008; Simpson et al., 2010). Por esse motivo, a relação entre o DM e a periodontite tem sido estudada em muitas populações durante os últimos anos (Emrich et al., 1991; Novak et al., 2008; Awartani, 2009).

Por mais de duas décadas, estudos epidemiológicos em diferentes populações, têm avaliado a relação entre o DM e a periodontite e corroboram a evidência de que a incidência e a gravidade da perda de inserção periodontal são mais altas em diabéticos (Löe, 1993). Por apresentarem altos índices de prevalência e incidência de DM tipo 2, comunidades de índios Pima no México e nos Estados Unidos, são avaliadas desde a década de 70. Em um dos primeiros estudos sobre a relação periodontia e DM, Emrich et al. (1991) avaliaram 1342 índios Pima de uma comunidade no Arizona e observaram que os índios diabéticos

apresentavam maior prevalência e gravidade de doença periodontal comparado aos índios não-diabéticos. Em 1998, Taylor et al. observaram a progressão da doença periodontal em indivíduos diabéticos comparado a indivíduos não-diabéticos, por um período de 2 anos. Após realizar ajustes para fatores de confundimento, os autores verificaram que a presença de DM estava positivamente correlacionada com a probabilidade de reabsorção óssea e que a progressão da periodontite foi 4.23 vezes maior nos indivíduos diabéticos. Novak et al. (2008) avaliaram o impacto do DM na extensão e gravidade da periodontite em indivíduos hispano-americanos portadores de DM tipo 2. Os autores observaram que os indivíduos com DM apresentavam maior gravidade e extensão de destruição periodontal que os indivíduos não-diabéticos, e que o DM também estava associado à maior formação de cálculo e perda dentária. Kaur et al. (2009) investigaram a associação entre a doença periodontal e o DM em 4288 indíviduos pomeranos, não-diabéticos e diabéticos tipo 1 e tipo 2. Os autores concluíram que havia uma associação entre a periodontite e a perda dentária e o DM tipo 1 e tipo 2. Preshaw et al. (2010) reportaram que indivíduos do Sri-Lanka com DM tipo 2 apresentavam um número de sítios significativamente maior com profundidade de sondagem (PS) $\geq$ 4 ou 5 mm, recessão gengival e sangramento à sondagem (SS). Susanto et al. (2011) avaliaram a associação entre a periodontite e o DM tipo 2 em uma população da Indonésia, que não havia sido estudada até o momento do estudo. Em concordância com os estudos acima descritos, os autores notaram que a prevalência e a gravidade da periodontite foram significativamente maiores em indivíduos diabéticos tipo 2 comparado aos indivíduos não-diabéticos. Lakschevitz et al. (2011), em uma revisão de literatura, analisaram a relação entre o DM e a periodontite. Os autores concluíram que diabéticos tipo 2 apresentavam um risco aumentado para doenças periodontais, e que pacientes com pobre controle glicêmico tinham risco aumentado para periodontite avançada. Botero et al. (2012) realizaram um estudo com 65 diabéticos e 81 não-diabéticos com o objetivo de avaliar a relação entre os níveis de glicose sanguínea e os parâmetros clínicos da doença periodontal. Após análise comparativa, foi observado que os não-diabéticos apresentavam maior número de dentes que os diabéticos. Além disso, diabéticos com periodontite tinham maior perda de inserção periodontal comparado aos não-diabéticos. Os autores concluíram ainda que a perda de inserção clínica periodontal foi aumentada devido à hiperglicemia em indivíduos diabéticos. Awuti et al. (2012)

demonstraram em uma população de 962 chineses, que a prevalência do DM foi significativamente maior em indivíduos com periodontite (71,3%) quando comparado a indivíduos sem periodontite (22,4%). Han et al. (2012), em um estudo tranversal, utilizaram a sonda da OMS (CPI – Índice periodontal comunitário) para classificar 4.118 indivíduos coreanos como apresentando ou não periodontite. Além disso, os indivíduos foram questionados a respeito do uso de cigarro e da presença de DM. Após ajustes para fatores de confundimento, os autores investigaram se o cigarro e o DM estavam associados à periodontite. Os autores verificaram que as prevalências totais de periodontite, tabagismo e auto-relato de DM em adultos coreanos foram 10,1%, 25,9% e 4,5%, respectivamente. Ao contrário dos estudos anteriores, surpreendentemente, a associação entre o DM e a periodontite não foi significativa e também não houve interações sinérgicas entre o tabagismo e o DM na periodontite. Os resultados sugeriram que apenas o cigarro apresentou um impacto significativo sobre a saúde periodontal de adultos coreanos.

1.3 Influência do controle glicêmico na periodontite

Um dos fatores mais relevantes no estudo da relação entre o DM e a periodontite consiste no controle glicêmico. Muitos estudos têm sugerido que a qualidade do controle glicêmico é um importante fator de risco para a gravidade da periodontite, demonstrando que pacientes com controle inadequado apresentavam doença periodontal mais avançada do que pacientes com bom controle (Tervonen; Knnuttila, 1986; Seppala; Ainamo, 1994; Lim et al., 2007; Awartani, 2009; Nesse et al., 2009; Chen et al., 2010). Estes achados se tornam mais importantes quando se leva em consideração o fato de que a associação entre a condição periodontal e glicêmica pode ter influencia mútua. Tem sido sugerido que o DM predispõe à doença periodontal enquanto a presença da periodontite pode intensificar a hiperglicemia em indivíduos diabéticos (Taylor, 1996; Janket et al., 2005) e aumentar os níveis de HbA1c em indivíduos não-diabéticos (Hayashida et al., 2009; Wolff et al., 2009). Este assunto será melhor abordado posteriormente no sub-item “Terapia periodontal em diabéticos e influência negativa da periodontite no controle glicêmico”.

Bacic et al. (1988) analisaram a influência da duração e controle do DM na periodontite, em diabéticos tipo 1 e 2. O estudo não demonstrou diferenças na gravidade da condição periodontal em relação ao nível de controle glicêmico da população estudada. Tervonen e

Karjalainen (1997), avaliando diabéticos tipo 1, observaram que o nível de saúde periodontal dos indivíduos com DM com bom ou moderado controle glicêmico era similar ao indivíduos não-diabéticos. Além disso, os autores não encontraram diferenças no número de bolsas periodontais ≥ 4 mm com SS entre indivíduos com controle glicêmico insatisfatório, moderado ou satisfatório. Kardesler et al. (2010) demonstraram que a PS, nível clínico de inserção e SS foram semelhantes em diabéticos bem-controlados (HbA1c <7%) e não-controlados (HbA1c ≥7%) com periodontite crônica.

Por outro lado, a maioria dos estudos demonstrou uma correlação positiva entre a gravidade da periodontite e o controle glicêmico insatisfatório. Tervonen e Knuuttila (1986) observaram que indivíduos com DM bem controlado apresentavam uma condição periodontal mais satisfatória quando comparado a indivíduos com DM não-controlado. Seppala e Ainamo (1994) avaliaram a condição periodontal de diabéticos tipo 1 em períodos de acompanhamento de 1 e 2 anos. Os autores verificaram que diabéticos não-controlados apresentavam maiores níveis de perda óssea alveolar quando comparado aos indivíduos controlados em 2 anos de acompanhamento. Lim et al. (2007) avaliaram a correlação entre os níveis de HbA1c e os parâmetros periodontais em diabéticos tipo 1 e tipo 2. Os resultados demonstraram que as porcentagens de sítios com SS e sítios com PS ≥ 5 mm estavam positivamente correlacionadas a um controle glicêmico inadequado (HbA1c >8%). Nesse et al. (2009) após uma análise de indivíduos com DM tipo 2, estabeleceram uma relação de dose-resposta entre a superfície periodontal inflamada e os níveis de HbA1c. Chen et al. (2010) analisaram a média de PS de boca-toda como uma possível variável preditora para níveis elevados de HbA1c. Foram realizados ajustes para fatores de confundimento como tabagismo, idade, gênero e índice de massa corpórea. O estudo demonstrou que os indivíduos com maior média de PS apresentavam maiores níveis de HbA1c, confirmando segundo os autores a hipótese inicial do estudo. Bandyopadhyay et al. (2010) utilizaram um modelo de regressão logística para avaliar a associação entre o controle glicêmico e a progressão da periodontite em uma população africana. Os resultados demonstraram que sítios periodontais de diabéticos não-controlados apresentavam maiores chances de progressão de doença quando comparado aos indivíduos bem-controlados. Recentemente, Morita et al. (2012) avaliaram a relação bidirecional entre a condição periodontal e o controle glicêmico. O estudo foi dividido em duas fases. A

primeira fase incluiu 5856 diabéticos com taxas de HbA1c ≥ 6,5%, sem bolsas ≥ 4 mm. O objetivo desta fase foi avaliar, em 5 anos, o risco de aparecimento de bolsas ≥ 4 mm nestes indivíduos. Foi observado que o risco para o desenvolvimento de bolsas periodontais nos diabéticos com HbA1c ≥ 6.5 foi 1.17 vezes maior. A segunda fase incluiu 6125 indivíduos com HbA1c <6,5% e objetivou avaliar a possível elevação da HbA1c, em 5 anos, em relação ao estado periodontal inicial. O risco relativo para o aparecimento de taxas de HbA1c ≥ 6,5% em indíviduos com bolsas entre 4-5 mm e ≥ 6 mm foi de 2,47 e 3,45 vezes maior, respectivamente. Assim, os autores concluíram que o risco para presença de doença periodontal estava associado aos níveis de HbA1c e o risco para elevação da HbA1c estava associado à presença de bolsas ≥ 4 mm.

1.4 Papel das citocinas e fatores relacionados à osteoclastogênse na periodontite e na periodontite associada ao DM

Embora já esteja bem estabelecido que a presença de patógenos é essencial para o desenvolvimento da doença periodontal, estudos têm demonstrado que a resposta do hospedeiro desempenha um papel crítico no curso desta doença (Graves, 2008). A resposta do hospedeiro a patógenos e a seus produtos na doença periodontal, é mediada pela liberação de uma série de biomarcadores inflamatórios, que podem afetar profundamente a natureza protetora ou destrutiva deste hospedeiro (Van Dyke; Serhan, 2003; Tatakis; Kumar, 2005). Dentre os vários mediadores inflamatórios envolvidos neste processo, as primeiras citocinas liberadas são da imunidade inata como, por exemplo, o fator de necrose tumoral (TNF)-α. Dentre muitas funções biológicas, o TNF-α apresenta participação no processo de migração celular, induzindo o aumento da regulação das moléculas de adesão e a produção de quimiocinas, as quais estão envolvidas na migração de células para os sítios infectados (Garlet et al., 2010). Além disso, o TNF-α apresenta capacidade para aumentar a produção de outras citocinas próinflamatórias da imunidade inata como a interleucina (IL)-β (Dinarello, 1996; Wajant et al., 1998; Garlet et al., 2007; Graves, 2008). Assim, em geral, as principais atividades biológicas destas citocinas da resposta imune inata são de caráter próinflamatório, incluindo o estímulo para a produção de moléculas de adesão, metaloproteinases, fatores relacionados à osteoclastogênese e outros mediadores próinflamatórios (Dinarello, 2009; Apostolaki et al., 2010; Gabay et al., 2010). Alguns

estudos avaliaram os níveis séricos de TNF-α em indivíduos diabéticos com periodontite (Iwamoto et al., 2001; Chen et al., 2010), enquanto outros trabalhos focaram nos níveis locais deste mediador na periodontite associado ao DM. Salvi et al. (1998) analisaram o fluido gengival de diabéticos e não-diabéticos com doença periodontal para a presença de TNF-α, prostaglandina (PGE)-2 e IL-1ß. Os resultados demonstraram níveis significativamente maiores de todas as citocinas no fluido gengival dos diabéticos quando comparado aos não-diabéticos. Duarte et al. (2007) analisaram a expressão de TNF-α e IL-1β em indivíduos diabéticos com periodontite, não-diabéticos com periodontite e periodontalmente e sistemicamente saudáveis. A expressão de IL-1β foi significativamente maior nos diabéticos quando comparado aos periodontalmente e sistemicamente saudáveis, enquanto não houve diferenças entre os grupos para a expressão de TNF-α. Venza et al. (2010) demonstraram uma alta expressão de TNF-α em sítios com periodontite crônica de diabéticos tipo 2 descompensados. Aspriello et al. (2010) compararam os níveis de TNF-α e IL-1β no fluido gengival de indivíduos com DM tipo 1 e tipo 2, na tentativa de identificar se havia diferenças no perfil destas citocinas nos diferentes tipos de DM. Os resultados demonstraram que diabéticos tipo 1 apresentavam níveis mais elevados de TNF-α e IL-1β e que existia uma correlação negativa entre os níveis desses marcadores e a duração do DM tipo 1. Ribeiro et al. (2011) compararam os níveis de TNF-α, IL-4, IL-23, IL-17 e interferon (INF)-γ no fluido gengival de indivíduos sistemicamente saudáveis comparado à diabéticos, ambos com periodontite crônica. Os resultados demonstraram níveis aumentados das citocinas próinflamatórias no fluido gengival de indivíduos com DM com periodontite crônica em relação aos não-diabéticos.

Após ocorrer a estimulação por um antígeno, células T+CD4, que tem papel fundamental na resposta imune mediada por células, podem se diferenciar em células efetoras T auxiliares (Th). As populações de células Th se dividem em sub-populações de Th1, Th2 e Th17 de acordo com os perfis de produção de citocinas que as mesmas apresentam (Tesmer et al., 2008; Zhu et al., 2010). Em geral, as células Th1 estão predominantemente relacionadas às respostas celulares e próinflamatórias, enquanto que células Th2 estão associadas com a resposta imune humoral e apresentam propriedades anti-inflamatórias (Jankovic et al., 2001; Garlet, 2010). Estudos sobre o perfil Th1/Th2 na patogênese das doenças periodontais observaram que citocinas como a IL-12 e o INF-γ estão associadas

principalmente a resposta Th1 e, dessa forma, conduzem para uma resposta imune mediada por células. Em contraste, a IL-4, IL-5, IL-10 e IL-13 estão envolvidas na resposta Th2 e promovem a imunidade humoral pela produção de fatores de crescimento e diferenciação para linfócitos B (Gemmell; Seymour, 2004). Até o presente momento, poucos estudos avaliaram as citocinas das respostas Th1 e Th2 em diabéticos com periodontite. Uma vez que o INF-γ e a IL-4 são as principais citocinas das respostas Th1 e Th2, respectivamente, as funções biológicas e o papel nas periodontites das mesmas serão descritos nos próximos parágrafos.

Entre as citocinas Th1, o INF-γ apresenta como principal função a ativação de macrófagos, tanto nas respostas imune inata como na mediada por células. O mesmo estimula a produção de uma série de mediadores próinflamatórios, como a IL-12 e o TNF-α. Números elevados de células produtoras de INF-γ nos tecidos periodontais e níveis elevados de INF-γ no fluido gengival têm sido associados com progressão da periodontite crônica (Ukai et al., 2001; Dutzan et al., 2009a). Górska et al. (2003) avaliaram a relação entre os parâmetros clínicos periodontais e as concentrações no tecido gengival das citocinas INF-γ, IL-4, IL-10, IL-1β, TNF-α e IL-2, em indivíduos com periodontite crônica avançada comparado à indivíduos periodontalmente saudáveis. As concentrações de INF-γ, IL1-β, TNF-α e IL-2 estavam significativamente maiores nas amostras de tecido gengival dos indivíduos com periodontite avançada. Honda et al. (2006) compararam o perfil de expressão gênica dos mediadores próinflamatórios INF-γ, IL-1β, TNF-α e IL-2 por meio da reação em cadeia da polimerase (PCR) na gengivite e na periodontite. Os autores verificaram que houve uma tendência para níveis aumentados de INF-γ e IL-1β na periodontite, enquanto que para o TNF-α e para a IL-2 não houve diferenças estatísticas. Dutzan et al. (2009a) analisaram os níveis de INF-γ em amostras de fluido gengival de indíviduos com periodontite crônica moderada e avançada. Foram detectados maiores quantidades totais e concentrações de INF-γ nas lesões periodontais ativas, quando comparado às inativas.

A IL-4, por sua vez, foi primeiramente descrita como um fator de crescimento de células B por ser um importante modulador da diferenciação de células Th2, intermediando a resposta humoral. Hart et al. em 1989, já haviam descrito a IL-4 como supressora de alguns

parâmetros de ativação de monócitos, e células B, e como tendo efeitos opostos ao INF-γ. A mesma é um potente inibidor de macrófagos e seu efeito anti-inflamatório resulta em parte, de sua eficiente inibição da produção de citocinas proinflamatórias como TNF-α, IL-1β, IL-6 e IL-8 (Te Velde et al., 1990). Estudos observaram que a ausência de IL-4 nos tecidos periodontais estava associada à atividade e progressão de doença, como, por exemplo, à evolução da gengivite para a periodontite (Shapira et al., 1992; Tsai et al., 2007). Pradeep et al. (2008) investigaram, em uma população Indiana, a relação entre condições clínicas periodontais e as concentrações de IL-4 no fluido gengival de sítios saudáveis, com gengivite e com periodontite crônica antes e após tratamento. Os resultados demonstraram maiores níveis de IL-4 em indivíduos saudáveis, níveis intermediários nos tratados e com gengivite e os menores níveis nos indivíduos com periodontite crônica. Os autores sugeriram que a presença dessa citocina estava associada com a remissão ou melhora da periodontite.

Evidências científicas têm proposto uma relação de antagonismo entre o INF-γ e a IL-4. Enquanto o INF-γ desempenha um papel próinflamatório, a IL-4 tenta compensar a função próinflamatória do INF-γ por meio de uma ação anti-inflamatória, sendo que o equilíbrio entre ambas citocinas pode ser capaz de regular a resposta imunoinflamatória nas doenças periodontais (Hart et al., 1989). Ukai et al. (2001) observaram que a proporção IL-4/INF-γ era menor em bolsas profundas do que em bolsas moderadas e rasas. Tsai et al. (2007) avaliaram pelo ensaio imunoenzimático (*Enzyme-linked immunosorbent assay* - ELISA), a presença de IL-4 e INF-γ no fluido gengival de indivíduos com periodontite crônica antes e após terapia periodontal não-cirúrgica. Os resultados demonstraram que a terapia periodontal foi capaz de reduzir a quantidade de INF-γ e aumentar a concentração de IL-4, sugerindo que uma baixa razão IL-4/INF-γ pode apresentar um papel importante na destruição dos tecidos periodontais. Shin et al. (2010) selecionaram indivíduos diabéticos tipo 2 com periodontite, sistemicamente saudáveis com periodontite e sistemicamente e periodontalmente saudáveis, objetivando quantificar a expressão de IL-4 e INF-γ no tecido gengival. Os autores concluíram que, independente da presença do DM, houve uma tendência para uma maior expressão de INF-γ nos grupos que apresentavam periodontite, e que a maior expressão de IL-4 foi observada no grupo dos indivíduos sadios.

Durante anos, a patogênese da periodontite foi descrita como um possível desequilíbrio entre os eixos Th1 e Th2 (Gemmell; Seymour, 2004). Recentemente, este conceito foi revisto devido a descoberta de uma nova população de células Th, antagônicas às células T regulatórias (T-reg), denominada Th17. As células Th17, se caracterizam especialmente pela produção da IL-17, a qual tem sido associada a inúmeras condições inflamatórias e autoimunes (Dong, 2008; Sallusto; Lanzavecchia, 2009). No ano de 2000, uma nova cadeia de citocinas foi descoberta, a p19, (Oppmann et al., 2000). Foi observado que essa cadeia forma heterodímero com a cadeia p40 da IL-12 e foi denominada IL-23. Langrish et al. (2005) demonstraram que a IL-23 expande e gera a produção das células T produtoras de IL-17, que por ser uma citocina próinflamatória que compartilha as ações biológicas da IL-1β e do TNF-α (Xu; Cao, 2010), tem sido apontada como um mediador importante na doença periodontal. Ohyama et al. (2009) analisaram e quantificaram por meio do teste de PCR em tempo real, os níveis das citocinas IL-23, IL-12, IL-17 e INF-γ em biópsias de tecido gengival de indivíduos com periodontite crônica moderada a avançada. Os resultados demonstraram níveis mais elevados de IL-23, IL-12 e IL-17 em lesões periodontais quando comparado aos tecidos sadios. Dutzan et al. (2009b) observaram que havia uma expressão exacerbada de IL-17 em lesões periodontais ativas quando comparado às lesões inativas bem como uma correlação positiva entre a expressão de IL-17 e o ligante do receptor do ativador do fator nuclear kappa-ß (RANKL). Cardoso et al. (2009) investigaram por meio de PCR, a presença de células Th17 na doença periodontal. Foram coletadas biópsias de tecido gengival e de osso alveolar de indivíduos saudáveis e portadores de periodontite para análise dos níveis de RNAm de diversas citocinas, incluindo IL-17 e IL-23 no tecido gengival e IL-17 e RANKL no osso alveolar. O estudo demonstrou altos níveis de IL-17 e IL-23 nos tecidos doentes, e indicou a presença de células Th17 nos tecidos gengivais dos indivíduos com periodontite. Foi possível observar ainda que a IL-17 e o RANKL foram expressos em níveis aumentados no osso alveolar dos indivíduos doentes em comparação aos baixos níveis encontrados nos indivíduos saudáveis.

Em relação ao papel das citocinas Th17 desempenhado em indivíduos com DM e periodontite, existem poucos relatos na literatura. Em 2011, um estudo do nosso grupo de pesquisa se propôs a avaliar em indivíduos sistemicamente e periodontalmente saudáveis, indivíduos sem nenhum fator de risco com periodontite, fumantes com periodontite e

diabéticos com periodontite os níveis de IL-17 em biópsias de tecido gengival. Foi observado que os níveis de IL-17 estavam maiores no grupo dos indivíduos diabéticos com periodontite quando comparado aos demais grupos (Duarte et al., 2011). Mais tarde, outro trabalho de nosso grupo de pesquisa avaliou os níveis de RNAm para diversos marcadores inflamatórios, dentre eles a IL-17 e a IL-23, por meio do PCR quantitativo. Os seguintes grupos de indivíduos foram avaliados: sistemicamente e periodontalmente saudáveis, sistemicamente saudáveis com periodontite, diabéticos com bom controle e pobre controle glicêmico, ambos com periodontite. Foi observado uma alta frequência de IL-17 nas amostras dos grupos com periodontite crônica, quando comparado ao grupo periodontalmente saudáveis (Duarte et al., 2012).

Em 1997, Simonet et al. isolaram uma glicoproteína capaz de regular o remodelamento ósseo, a osteoprotegerina (OPG). A OPG pertence à super-família do TNF e atua no ambiente extracelular inibindo o processo de reabsorção óssea. Um ano mais tarde, Yasuda et al. (1998) identificaram outros dois membros da superfamília do TNF, também muito importantes no processo de reabsorção óssea, o ativador do fator nuclear kappa-ß (RANK) e seu ligante, RANKL. Por meio da interação RANK/RANKL ocorre a diferenciação e atividade de osteoclastos. Por outro lado, a OPG é capaz de bloquear a interação RANK/RANKL, por meio de sua ligação ao RANKL e, desta forma, inibir o processo de reabsorção óssea (Troen, 2003). Estudos têm demonstrado que citocinas próinflamatórias são capazes de induzir a expressão de RANKL, gerando assim uma importante interação entre as células ósseas e as células imunológicas. Boyce et al. (2005) demonstraram que o TNF-α foi capaz de induzir a expressão de RANKL, enquanto Sato et al. (2006) observaram que a IL-17 induziu a expressão de RANKL.

Níveis aumentados de RANKL e reduzidos de OPG têm sido encontrados na doença periodontal (Vernal et al., 2004; Lu et al., 2006; Bostanci et al., 2007). Por esse motivo, foi sugerido que essas moléculas poderiam ocupar um papel importante na destruição do tecido ósseo alveolar em casos de periodontites. Teng et al. (2000), utilizando um modelo animal, sugeriram o envolvimento de RANKL e OPG na patogênese das doenças periodontais. Da mesma forma, estudos em humanos demonstraram que citocinas inflamatórias, presentes no fluido gengival de pacientes com periodontite, são capazes de modular a produção de

RANKL e, consequentemente, a reabsorção óssea na doença periodontal. Vernal et al. (2004) avaliaram os níveis de RANKL no fluido gengival de indivíduos com periodontite comparado à indivíduos periodontalmente saudáveis. Os autores observaram maiores níveis de RANKL no fluido gengival de indivíduos com periodontite, demonstrando haver uma correlação positiva entre RANKL e a perda óssea alveolar na doença periodontal. Mogi et al. (2004) analisaram pelo teste ELISA a presença de RANKL e OPG no fluido gengival de indivíduos periodontalmente saudáveis, com periodontite leve, moderada ou avançada. Os resultados demonstraram altos níveis de RANKL e baixos níveis de OPG em indivíduos com periodontite, que também apresentaram a maior proporção RANKL/OPG quando comparado aos indivíduos saudáveis. Lu et al. (2006) objetivaram quantificar a proporção RANKL/OPG no fluido e tecido gengival de indivíduos com periodontite, e avaliar uma possível correlação com a gravidade da doença. Os resultados demonstraram níveis mais elevados de RANKL e mais baixos de OPG no fluido gengival de sítios doentes, sugerindo uma influência negativa de RANKL na gravidade de doença. A avaliação do tecido gengival demonstrou células positivas para RANKL significativamente distribuídas nos tecidos com periodontite quando comparado as amostras de tecidos saudáveis. Em relação a OPG, poucas células foram marcadas mesmo no tecido sadio. Bostanci et al. (2007) compararam por meio do teste ELISA, os níveis de RANKL e OPG no fluido gengival de indivíduos saudáveis, com gengivite, periodontite crônica, periodontite agressiva generalizada e periodontite crônica submetidos a terapia com imunossupressores. Os resultados demonstraram baixos níveis de RANKL e RANKL/OPG nos indivíduos saudáveis e com gengivite e altos níveis nos indivíduos com os diferentes tipos de periodontites. Os autores encontraram ainda uma correlação positiva entre a proporção RANKL/OPG e a PS e o nível de inserção clínica. Belibasakis e Bostanci (2012), em uma revisão de literatura, concluíram que o aumento da razão RANKL/OPG pode servir como um biomarcador que denota a ocorrência da periodontite, mas não necessariamente prediz a atividade de doença.

Até o momento, poucos estudos focaram a influência do sistema RANKL/OPG em indivíduos com periodontite e DM. Duarte et al. (2007) detectaram altos níveis de RNAm para RANKL e baixos níveis para OPG em indivíduos diabéticos tipo 1 com periodontite. Recentemente, dois estudos do nosso centro de pesquisa avaliaram no fluido e no tecido

gengival, o papel desempenhado por RANKL e OPG em diferentes tipos de grupos de indivíduos. Ribeiro et al. (2011) avaliaram os níveis de RANKL e OPG no fluido gengival de indivíduos sistemicamente saudáveis com periodontite crônica comparado a indivíduos diabéticos tipo 2 com periodontite crônica. Os autores observaram níveis aumentados de RANKL no fluido gengival de indivíduos com DM tipo 2 comparado aos indivíduos não-diabéticos com periodontite crônica. Duarte et al. (2012) avaliaram os níveis de RNAm para RANKL e OPG, em amostras gengivais de indivíduos periodontalmente e sistemicamente saudáveis, sistemicamente saudáveis com periodontite crônica, diabéticos com pobre e bom controle glicêmico com periodontite crônica. Os resultados demonstraram níveis significativamente altos de RANKL nos grupos com periodontite crônica, quando comparado ao grupo sistemicamente e periodontalmente saudáveis.

1.5 Terapia periodontal em diabéticos e a influência negativa da periodontite no controle glicêmico

Nas últimas décadas, muitos estudos avaliaram a aplicação da terapia periodontal básica (raspagem e alisamento radicular [RAR] associada ao controle do biofilme supragengival) em indivíduos com periodontite, pertencentes ou não à grupos de risco. Em relação aos diabéticos, tem sido observado que a terapia periodontal básica promove benefícios clínicos muitas vezes semelhantes aos observados em indivíduos não-diabéticos (Grossi et al., 1997; Simpson et al., 2010). Entretanto, os benefícios do tratamento periodontal no controle glicêmico de diabéticos ainda não foram totalmente esclarecidos (Janket et al., 2005; Darré et al., 2008; Teeuw et al., 2010). Dentro da linha de pesquisa sobre os efeitos sistêmicos da periodontite, algumas investigações têm sugerido que diabéticos com infecção periodontal apresentam maior dificuldade para controlar a glicemia em relação aos diabéticos sem doença periodontal (Choi et al., 2011). Diversos estudos testaram essa hipótese acompanhando os efeitos do tratamento periodontal sobre os níveis de HbA1c em diabéticos (Rodrigues et al., 2003; Janket et al., 2005; Promsudthi et al., 2005; Faria-Almeida et al., 2006; Navarro-Sanchez et al., 2007; Darré et al., 2008; Santos et al., 2009). Faria-Almeida et al. (2006) avaliaram os resultados do tratamento periodontal básico, em indivíduos não-diabéticos comparado à diabéticos tipo 2, em relação aos parâmetros clínicos periodontais. Os autores observaram melhoras em todos os parâmetros clínicos, em

3 e 6 meses, sem diferenças entre os grupos. Foi observado uma melhora no nível de HbA1c, confirmando uma resposta metabólica positiva ao tratamento periodontal nos indivíduos diabéticos. Similarmente, Navarro-Sanchez et al. (2007) compararam os resultados clínicos do tratamento periodontal básico em pacientes diabéticos e não-diabéticos, bem como seus efeitos nos níveis de IL-1β e TNF-α no fluido gengival. Os resultados demonstraram melhora para todos os parâmetros clínicos em ambos os grupos sem diferenças entre os grupos. O nível de HbA1c reduziu significativamente em 3 e 6 meses pós-terapia nos indivíduos diabéticos. Os autores constataram ainda que ambos os grupos reduziram os níveis de IL-1β e TNF-α pós-terapia, sem diferenças entre os grupos. O'Connell et al. (2008) compararam os resultados do tratamento periodontal básico somente à terapia básica associada à doxiciclina em diabéticos tipo 2. Foi observado que ambas as terapias reduziram significativamente, em 3 meses, a PS e os níveis de alguns marcadores inflamatórios séricos, incluindo a IL-6 e a IL-12. Koromantzos et al. (2011) avaliaram o controle glicêmico de 60 indivíduos diabéticos tipo 2 com periodontite moderada-avançada que receberam terapia periodontal. Trinta indivíduos receberam RAR não-cirúrgica, enquanto os demais não receberam tratamento por 6 meses. Os resultados demonstraram que todos os parâmetros clínicos melhoraram significativamente nos pacientes submetidos ao tratamento, o que contribuiu fortemente, segundo os autores, para melhorar o controle glicêmico nos indivíduos diabéticos. Sun et al. (2011) avaliaram os resultados da terapia periodontal nos níveis glicêmicos e na redução de marcadores inflamatórios em indivíduos diabéticos tipo 2. Os autores concluíram que a terapia periodontal foi capaz de reduzir os níveis de marcadores inflamatórios bem como melhorar o controle glicêmico dos indivíduos, 3 meses pós-terapia. Moeintaghavi et al. (2012) examinaram os efeitos do tratamento periodontal não-cirúrgico no controle glicêmico de indivíduos diabéticos tipo 2. Quarenta indivíduos diabéticos com periodontite crônica foram divididos aleatoriamente em dois grupos: RAR ou ausência de tratamento periodontal. Os parâmetros clínicos periodontais, glicemia em jejum e HbA1c foram avaliados no início do estudo e em 3 meses pós-terapia. Os autores concluíram que o tratamento periodontal foi capaz de melhorar o controle metabólico dos indivíduos diabéticos.

Com o objetivo de elucidar os efeitos do tratamento periodontal no controle glicêmico e entender os efeitos da infecção periodontal na glicemia, algumas meta-análises foram realizadas na última década. Janket et al. (2005) avaliaram 10 estudos intervencionais e encontraram, em média, uma diminuição de 0,38% de redução de HbA1c considerando os 10 estudos, 0,66% considerando os estudos realizados em diabéticos tipo 2 e 0,71% para os que incluíram antibióticos na terapia. Entretanto, nenhuma destas diminuições foram estatisticamente significantes. Darré et al. (2008), após uma ampla revisão de literatura, realizaram uma meta-análise em 9 estudos clínicos controlados e randomizados. Os autores sugeriram que o tratamento periodontal contribuiu para a melhora do controle glicêmico em diabéticos tipo 1 e 2. Teew et al. (2010), por sua vez, incluíram em sua meta-análise apenas estudos realizados em diabéticos tipo 2. Os autores observaram que o tratamento periodontal levou a uma melhora no controle glicêmico 3 meses pós-terapias. Simpson et al. (2010) sugeriram que existe uma moderada, mas significante, melhora no controle glicêmico após o tratamento periodontal em indivíduos diabéticos tipo 2. Entretanto, segundo os autores, estudos clínicos futuros bem delineados ainda são necessários para confirmar tal hipótese.

1.5.1 Tratamento periodontal de boca-toda em curto período associado ou não ao uso de clorexidina

Estudos têm sugerido que sítios periodontais tratados poderiam ser re-infectados por patógenos de sítios ainda não tratados quando aplicada a RAR por quadrante, realizada em sessões com intervalos de 1 a 3 semanas (Quirynen et al., 1995; Apatzidou; Kinane, 2004a; Zijnge et al., 2010). Esta re-infecção poderia ocorrer por uma possível translocação de bactérias de um sítio para outro na cavidade oral. Na tentativa de impedir esta re-infecção, alguns trabalhos sugeriram a utilização da RAR de boca-toda em um curto período, isto é, entre 24-48 horas. Apatzidou e Kinane (2004a) avaliaram os possíveis benefícios clínicos da RAR de boca-toda em curto período sobre a RAR convencional por quadrante no tratamento de indivíduos com periodontite crônica. Foi observado que ambas as terapias promoveram melhoras nos parâmetros clínicos, em 3 e 6 meses pós-terapias. Entretanto, o estudo não demonstrou diferenças entre os grupos para nenhum dos parâmetros clínicos avaliados. Somente para os sítios mais profundos (>7mm) houve um significante maior

ganho de inserção relativo para o grupo da RAR de boca-toda em curto período em 6 meses pós-terapias. Subsequentemente, esse mesmo grupo de pesquisa investigou nestes indivíduos os beneficios microbiológicos e imunológicos de ambas terapias. Assim, o estudo de Apatzidou e Kinane (2004b) avaliou, por meio de PCR, a presença de patógenos periodontais (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia, Treponema denticola* e *Tannerella forsythia*) no biofilme subgengival dos sítios mais profundos de cada quadrante (>7mm) após ambas terapias. Os resultados demonstraram que as duas terapias levaram a reduções significativas dos patógenos avaliados e a RAR de boca-toda em curto prazo não demonstrou benefícios adicionais sobre a RAR convencional. Em relação a avaliação imunológica, Apatzidou e Kinane (2004c) realizaram coleta sanguínea para avaliação da atividade de anticorpos (mensurada pela dissociação de tiocinato) contra os patógenos analisados no estudo anterior. As coletas sanguíneas foram realizadas duas vezes por semana durante as 4 semanas de tratamento, 3 e 6 meses pós-terapias. Os resultados demonstraram que ambas as terapias aumentaram a atividade de anticorpos, mas sem diferenças entre os grupos.

Koshy et al. (2005), em um estudo clínico randomizado, avaliaram os efeitos do debridamento com ultrassom por quadrante, debridamento de boca-toda com ultrassom em sessão única sob irrigação com iodo povidine e debridamento de boca-toda com ultrassom em sessão única sob irrigação com água. Os autores concluíram que os debridamentos de boca-toda em sessão única apresentaram benefícios modestos sobre a RAR por quadrante no tratamento da periodontite. Wennström et al. (2005) avaliaram o debridamento de boca-toda com ultrassom em sessão única comparado à RAR por quadrante com instrumentos manuais. Não foram encontradas diferenças entre os grupos de tratamento para nenhum parâmetro clínico. Zhao et al. (2005) analisaram os efeitos da RAR de boca-toda em curto prazo, comparado à RAR por quadrante, no tratamento de indivíduos com periodontite crônica. Os resultados demonstraram melhoras em todos os parâmetros clínicos periodontais para ambos os grupos de terapia, em 3 e 6 meses, sem diferenças entre os grupos em nenhum dos tempos. Tomasi et al. (2006) demonstraram que não houve diferença na recorrência de sítios doentes entre o debridamento de boca-toda com ultrassom em sessão de uma hora e a RAR por quadrante, em um tempo de acompanhamento de 12 meses. Similarmente, Jervøe-Storm et al. (2006) não demonstraram diferenças entre a RAR

de boca-toda em curto prazo e a RAR por quadrante para nenhum parâmetro clínico periodontal, em 3 e 6 meses pós-terapias. Mais tarde, o mesmo grupo de pesquisadores avaliou os efeitos de ambas terapias nos aspectos microbiológicos (Jervøe-Storm et al., 2007). Os seguintes patógenos periodontais foram avaliados e quantificados por PCR em tempo real: *A. actinomycetemcomitans, Fusobacterium nucleatum ssp., P. gingivalis, P.intermedia, T. denticola* e *T. forsythia*. Os resultados foram similares para ambas as terapias nas reduções das contagens totais dos patógenos, sem diferenças entre os grupos de terapias em todos os tempos de acompanhamento (Jervøe-Storm et al., 2007). Knöfler et al. (2007) avaliaram 37 indivíduos portadores de periodontite crônica por um período de 12 meses após RAR de boca-toda em curto período (17 indivíduos) ou RAR por quadrante (20 indivíduos). Os resultados demonstraram que ambas as terapias apresentaram melhoras na PS, SS e nível clínico de inserção sem diferenças entre os grupos. Zijnge et al. (2010) demonstraram que a RAR de boca-toda em curto prazo e a RAR por quadrante apresentaram resultados clínicos e microbiológicos similares, em 3 meses pós-terapias. Recentemente, Knöfler et al. (2011) analisaram, por PCR, a presença de 4 patógenos periodontais (*A. actinomycetemcomitans, P. gingivalis, T. forsythia* e *T. denticola*) antes e em 12 meses após o tratamento de indivíduos com periodontite crônica por meio de RAR por quadrante ou RAR de boca-toda em curto prazo. De acordo com os resultados, ambas as terapias promoveram efeitos similares nos níveis dos patógenos avaliados.

Tendo em vista que a RAR por quadrante ou de boca-toda em curto prazo atinge somente os sítios periodontias, e tendo conhecimento de que outros reservatórios da cavidade oral (ex.: língua, tonsilas, mucosa, etc) também estão contaminados por bactérias associadas à doença periodontal, alguns estudos sugeriram a associação do uso de antimicrobianos locais para evitar a translocação de bactérias destes sítios para sítios tratados. O digluconato de clorexidina, um anti-séptico de amplo espectro de ação e substantividade, foi sugerido como um potencial agente adjunto a terapia periodontal básica (Quirynen et al., 1995; Faveri et al., 2006; Feres et al., 2009). Segundo Quirynen et al. (1995), a clorexidina, poderia interferir na recolonização subgengival bacteriana de bolsas já raspadas por manter baixos níveis de biofilme supragengival e por atingir reservatórios bacterianos não-dentais presentes na cavidade oral que não seriam alcançados pela RAR. Sendo assim, os autores sugeriram a associação da RAR de boca-toda em curto prazo à uma extensa aplicação oral

de clorexidina, protocolo denominado desinfecção de boca-toda em estágio único. Os resultados de estudos do mesmo centro de pesquisas (Quirynen et al., 1995; Quirynen et al., 2000), demonstraram algumas vantagens clínicas e microbiológicas do uso da clorexidina 0,2% por 14 e 60 dias durante a fase inicial de cura (primeiro e segundo meses) após o protocolo de desinfecção de boca-toda, entretanto, esses benefícios não foram mantidos até o quarto e oitavo meses pós-terapia. Mongardini et al. (1999) compararam a utilização do protocolo de desinfecção de boca-toda com a RAR por quadrante no tratamento de indivíduos com periodontite crônica inicial (16 indivíduos) e avançada (24 indivíduos). Os autores observaram uma redução significativa na PS e um ganho de inserção, em 8 meses pós-terapia, para o grupo que recebeu o protocolo de desinfecção de boca-toda comparado ao grupo que recebeu a RAR por quadrante. O mesmo grupo de pesquisadores avaliou também os benefícios microbiológicos dessas terapias por meio de cultura, realizando coletas de sítios periodontais, mucosa, língua e saliva (Quirynen et al., 1999). Segundo os autores, comparado à terapia de RAR por quadrante, o protocolo de desinfecção de boca-toda em estágio único resultou em benefícios microbiológicos adicionais, apresentando maiores reduções nas proporções de espiroquetas e organismos móveis subgengivais, e uma significante redução na quantidade de patógenos periodontais, como, por exemplo, a eliminação de *P. gingivalis.* De Soete et al. (2001) avaliaram os efeitos microbiológicos do protocolo de desinfecção de boca-toda comparado à RAR por quadrante no tratamento de indivíduos com periodontite crônica ou agressiva. Trinta espécies bacterianas foram analisadas pelo teste “checkerboard DNA-DNA hybridization”. Os resultados demonstraram que ambas as terapias resultaram em importantes reduções nos níveis e nas freqüências de espécies patogênicas, em um período de 8 meses. Entretanto, o protocolo de desinfecção de boca-toda apresentou benefícios adicionais, principalmente no grupo com periodontite agressiva, no qual a redução de *P. gingivalis* e *T. forsythia* ficou abaixo do nível de detecção. Em 2006, Quirynen et al., na tentativa de elucidar se os efeitos benéficos do protocolo de desinfecção de boca-toda resultavam do uso dos anti-sépticos orais ou da RAR em um curto prazo (até 24 horas), avaliaram alguns protocolos terapêuticos: RAR em intervalo de duas semanas, RAR em 2 dias consecutivos, desinfecção de boca-toda com clorexidina por 2 meses, desinfecção de boca-toda com fluoreto de amina por 2 meses, desinfecção de boca-toda com clorexidina por 2 meses seguido de fluoreto de amina por

outros 6 meses. Todas as terapias demonstraram benefícios clínicos em até 8 meses pós-terapias, sem diferenças significativas entre os grupos. Os autores concluíram que os benefícios obtidos pelo protocolo de desinfecção de boca-toda pode ser atribuído em parte pelo uso dos anti-sépticos orais e em parte pelo tratamento em um curto prazo, e que em relação aos outros tipos de terapias o protocolo de desinfecção de boca-toda apresenta benefícios modestos. Swierkot et al. (2009), comparando a RAR de boca-toda em curto prazo *versus* o protocolo de desinfecção de boca-toda em não-diabéticos com periodontite crônica, não demonstraram diferenças significativas entre ambos tratamentos em 4 e 8 meses pós-terapias. Stratul et al. (2010) avaliaram o uso da clorexidina 0,2% por 3 meses após RAR de boca-toda em sessão única. Os autores concluíram que o uso da clorexidina apresentou modestas, mas significantes melhoras nos aspectos clínicos, em 3 meses pós-terapia. Aimetti et al. (2011) analisaram clinica- e microbiologicamente, em 6 meses, o uso do protocolo de desinfecção de boca-toda no tratamento de indivíduos com periodontite agressiva generalizada. Os resultados demonstraram uma melhora significativa em todos os parâmetros clínicos e, as porcentagens de sítios moderados e profundos livres de patógenos foram de 40% e 27%, respectivamente pelo teste de PCR. Os autores sugeriram o protocolo de desinfecção de boca-toda como uma terapia viável no tratamento da periodontite agressiva generalizada. Entretanto, esse estudo não foi controlado.

Revisões sistemáticas sugeriram que o protocolo de desinfecção de boca-toda em estágio único apresenta vantagens modestas sobre a RAR por quadrante no tratamento da periodontite crônica e nenhuma vantagem sobre a RAR de boca-toda em curto prazo (Eberhard et al., 2008; Lang et al., 2008). Em uma revisão sistemática, Eberhard et al. (2008) analisaram a efetividade do protocolo de desinfecção de boca-toda, da RAR de boca-toda em curto prazo e da RAR por quadrante. Os autores observaram apenas benefícios modestos do protocolo de desinfecção de boca-toda sobre as demais terapias. Similarmente, Farman e Joshi (2008) em sua revisão sistemática também não demonstraram vantagens clínicas do protocolo de desinfecção de boca-toda sobre a RAR de boca-toda ou por quadrante. A revisão de Lang et al. (2008) sugeriu que tanto a RAR por quadrante quanto a RAR de boca-toda em curto prazo e o protocolo de desinfecção de boca-toda são modalidades terapêuticas que podem ser recomendadas para o tratamento inicial de pacientes com periodontite crônica.

Especialmente em um grupo susceptível a infecção como os diabéticos, a RAR de boca-toda poderia apresentar algumas vantagens em relação a RAR por quadrante, como a diminuição no número de visitas ao consultório e uma abrupta redução na infecção bacteriana (Eberhard et al., 2008; Lang et al., 2008). Entretanto, poucos estudos controlados avaliaram o uso da RAR em curto prazo com ou sem clorexidina em diabéticos. Rodrigues et al. (2003) observaram reduções na PS, SS, supuração e acúmulo de placa e na taxa de HbA1c em 3 meses após o tratamento de indivíduos diabéticos tipo 2, utilizando RAR de boca-toda em único estágio. O'Connell et al. (2008) demonstraram que a RAR, em 24 até 36 horas utilizando instrumentos manuais e dispositivos ultrassônicos, melhorou todos os parâmetros clínicos em indivíduos diabéticos tipo 2, em 3 meses pós-terapia. Da Cruz et al. (2008) utilizaram um modelo de tratamento de RAR de boca-toda em uma sessão de aproximadamente 2 horas, em indivíduos diabéticos tipo 2 que faziam reposição de insulina. O estudo demonstrou melhoras siginificativas nos parâmetros clínicos periodontais, entretanto, não houve diferenças nos níveis de HbA1c em 3 meses pós-terapia. Um estudo prévio de nosso grupo de pesquisa comparou a RAR por quadrante e a RAR de boca-toda em curto prazo no tratamento de diabéticos tipo 2 com periodontite crônica. Os resultados demonstraram melhoras similares para os parâmetros clínicos e glicêmicos para ambas as terapias (Santos et al., 2009).

Em indivíduos diabéticos, não há na literatura dados suficientes para confirmar os efeitos clínicos e glicêmicos do uso da clorexidina em associação à RAR por quadrante ou em curto prazo. Grossi et al. (1997) compararam os efeitos da RAR com ultrassom e curetas associada à cinco tipos de protocolos para tratamento de indivíduos diabéticos tipo 2: uso tópico de água e doxiciclina sistêmica (100 mg por 2 semanas), uso tópico de clorexidina 0,12% e doxiciclina sistêmica (100 mg 2 vezes por semana), uso tópico de iodo povidine e doxiciclina sistêmica (100 mg 2 vezes por semana), uso tópico de clorexidina 0,12% e placebo e uso tópico de água e placebo. O estudo demonstrou que todos os protocolos de terapia apresentaram benefícios clínicos, e que os grupos que utilizaram a doxiciclina apresentaram as maiores reduções nas PS e nos níveis de HbA1c, em 3 meses pós-terapias. Madden et al. (2008) avaliaram os efeitos de dois protocolos distintos de terapia periodontal no controle glicêmico de 42 diabéticos tipo 2, em 6 e 8 meses pós-terapia. O primeiro protocolo consistia em RAR e instruções de higiene bucal em duas ocasiões com um

intervalo de seis meses, denominado terapia mínima (TM). O segundo protocolo consistia em RAR e instruções de higiene bucal a cada dois meses associado à bochechos de clorexidina 0,12% duas vezes ao dia, denominado terapia freqüente (TF). Os resultados demonstraram que indivíduos submetidos à TF apresentaram melhoras superiores nos aspectos clínicos periodontais quando comparados aos submetidos à TM. Em relação ao controle glicêmico, diabéticos com níveis de HbA1c ≥ 9% obtiveram maiores perdas de inserção quando comparados aos diabéticos com níveis de HbA1c < 9%. Além disso, os autores não observaram diferenças nas alterações dos níveis de HbA1c em diabéticos que receberam RAR associada aos bochechos de clorexidina, quando comparado aqueles que receberam somente a RAR. Em relação ao uso do protocolo de desinfecção de boca-toda em estágio único apenas o estudo não-controlado de Schara et al. (2006) demonstrou melhoras clínicas e no controle glicêmico de diabéticos tipo 1 tratados pelo protocolo.

2. PROPOSIÇÃO

Esse trabalho foi dividido em quatro estudos, cujos objetivos foram:

ESTUDO 1 – Comparar os níveis de fatores relacionados à osteoclastogênese (RANKL e OPG) no fluido gengival de indivíduos com periodontite crônica portadores de DM tipo 2 bem controlados ou com controle glicêmico insatisfatório, antes, 3 e 6 meses após terapia periodontal básica.

ESTUDO 2 - Comparar os níveis de citocinas (TNF-α, INF-γ, IL-4, IL-17 e IL-23) no fluido gengival de indivíduos com periodontite crônica portadores de DM tipo 2 bem controlados ou com controle glicêmico insatisfatório, antes, 3 e 6 meses após terapia periodontal básica.

ESTUDO 3 - Avaliar, em 12 meses, os efeitos da RAR de boca-toda em curto prazo (24 horas), comparativamente à RAR por quadrante, nos parâmetros clínicos e nos níveis de biomarcadores em diabéticos tipo 2 com periodontite crônica.

ESTUDO 4 - Avaliar, em 12 meses, os efeitos clínicos e glicêmicos da aplicação extensa de clorexidina associada à RAR de boca-toda em curto prazo (24 horas), protolocolo denominado desinfecção de boca-toda, em diabéticos tipo 2 com periodontite crônica.

3. ESTUDO 1

Receptor activator of NF-KB ligand /osteoprotegerin ratio in sites of chronic periodontitis of poorly-controlled and well-controlled type 2 diabetic subjects - J Periodontol. 2010 Oct;81(10):1455-65.

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ABSTRACT

Objective: The aim of this study was to evaluate the levels of osteoclastogenesis-related factors [soluble receptor activator of NF-$_{K}$B ligand (sRANKL) and osteoprotegerin (OPG)] in gingival crevicular fluid (GCF) from poorly- and well-controlled type 2 diabetic subjects with chronic periodontitis before and after periodontal therapy.

Methods: Eighteen well-controlled [glycated hemoglobin (HbA1c) levels ≤ 8%] and 20 poorly-controlled (HbA1c levels > 8%) diabetic subjects were enrolled in this study. All subjects were submitted to non-surgical periodontal therapy. GCF sampling and clinical periodontal parameters were assessed at baseline and 3 and 6 months post-therapy. Total amounts and concentrations of sRANKL and OPG in the GCF were analyzed by enzyme linked immunosorbent assay (ELISA).

Results: The total amount and concentration of sRANKL and the ratio RANKL/OPG were higher in poorly- than well-controlled subjects at baseline and 3 and 6 months post-therapy ($p<0.05$). In addition, the RANKL/OPG ratio decreased in well-controlled ($p<0.05$), but not in poorly-controlled subjects ($p>0.05$), at 3 months post-therapy. Almost all clinical parameters improved significantly for both groups post-treatment ($p<0.05$).

Conclusions: The RANKL/OPG ratio in untreated and treated periodontitis sites may be negatively influenced by a poor glycemic control in type 2 diabetic subjects.

Key-words: diabetes mellitus; chronic periodontitis; root planing; RANK Ligand; osteoprotegerin.

Sentence summary: The RANKL/OPG ratio in untreated and treated periodontitis sites may be negatively influenced by a poor glycemic control in type 2 diabetic subjects.

INTRODUCTION

Type 2 diabetes mellitus (DM) is the most prevalent type of DM among middle-aged subjects, characterized by impairment of insulin function in tissues and defects in pancreatic insulin secretion.[1] Insulin resistance and deficiency give rise to a hyperglycemic state that triggers the development of DM complications. Considerable evidence supports DM as a risk factor for periodontal diseases, since the incidence, progression and severity of periodontal diseases is higher in subjects with type 2 DM than those without DM.[2-3] In addition, it seems that periodontitis is even more exacerbated among poorly-controlled diabetic individuals.[4-5]

Alveolar bone loss, which is mediated by host immune response against biofilm accumulation, is one of the most important hallmarks of periodontitis.[6-8] Therefore, the current emergence of osteoimmunology has increased interest in defining the mechanisms underlying bone resorption in periodontal diseases. Among other mechanisms, bone remodeling is coordinated by the interaction between the receptor activator of NF-$_K$B (RANK), its receptor activator of NF-$_K$B ligand (RANKL) and osteoprotegerin (OPG), whose expressions are tightly regulated by inflammatory mediators and bacterial products.[7,9] Stromal, osteoblastic and activated T cells express RANKL in a bound membrane form, linking T-cell-mediated immunity and bone remodeling. Both transmembrane and soluble RANKL (sRANKL) forms can interact with RANK on

progenitors of monocytes/macrophages precursors, signaling mature osteoclast differentiation and activation. OPG, secreted by osteoblasts and bone marrow stromal cells, interrupts RANK-RANKL interplay by binding to membrane-bound and sRANKL. Therefore, RANKL/OPG imbalances have been related to several diseases, including osteoporosis, rheumatoid arthritis, metastatic bone cancer and periodontitis.[6-9]

In general, studies agree that the RANKL/OPG ratio is higher in sites with periodontal diseases compared to periodontally-healthy ones and, therefore, may play a role in modulating the localized bone loss in periodontitis.[10-15] To date, few studies have focused on the role of the RANKL-OPG system in subjects with periodontitis and DM.[16-17] In addition, no studies have evaluated so far the influence of glycemic control on the levels of these osteoclastogenesis-related factors in sites with periodontitis in subjects with type 2 DM. Therefore, the aim of this study was to compare the levels of sRANKL and OPG, as well as their ratio, in the gingival crevicular fluid (GCF) from poorly-controlled and well-controlled type 2 diabetic subjects with chronic periodontitis before and after non-surgical periodontal therapy. Our hypothesis is that a poor glycemic control may be associated with an increased RANKL/OPG ratio and may jeopardize the reduction of this ratio in response to non-surgical periodontal therapy in type 2 diabetic subjects.

MATERIAL AND METHODS

Subject population

Thirty-eight subjects (age range: 40 to 70 years) diagnosed with type 2 DM and chronic periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University, from July 2007 until March 2008. Detailed medical and dental records were obtained. Subjects who fulfilled the following inclusion/exclusion criteria were invited to participate in the study. All eligible subjects were thoroughly informed of

the nature, potential risks and benefits of their participation in the study and signed their informed consent. This study protocol was previously approved by Guarulhos University's Ethics Committee in Clinical Research (#100/2007).

Inclusion and exclusion criteria

Data concerning the duration of DM and medications were retrieved from the medical records of the subjects at the beginning of the study. All subjects had presented type 2 DM diagnosis for at least 5 years and were under insulin supplementation, diet regime and/or oral hypoglycemic agents. Subjects were diagnosed with generalized chronic periodontitis, based on the clinical and radiographic criteria proposed by the 1999 World Workshop for Classification of Periodontal Diseases and Conditions.[18] All subjects should present > 30 years old, had at least 15 teeth excluding third molars and teeth with advanced decay indicated to exodontias, and more than 30% of the sites had probing depth (PD) and clinical attachment level (CAL) ≥ 5 mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking, smoking within the past 5 years, periodontal or/and antibiotic therapies during the previous 6 months, use of mouth rinses containing antimicrobials in the preceding 2 months, any systemic condition (except DM) that could affect the progression of periodontal disease (e.g. immunological disorders, osteoporosis) and long-term administration of anti-inflammatory and immunosuppressive medications. Subjects with periapical pathology, orthodontic appliances, multiple systemic complications of DM and under hormone replacement, calcitonin and alendronate therapies were also excluded from the study.

Blood analyses and experimental groups

A single laboratory (Guarulhos University Clinical Analysis Laboratory) performed all blood analyses. Blood samples were taken for each subject at baseline and 3 and 6 months

post-therapy. The fasting plasma glucose (FPG), measured using the glucose oxidase method, was expressed in milligrams per deciliter (mg/dl) and presented a normal healthy range of 60 - 110 mg/dl. Glycated hemoglobin (HbA1c), measured by high-performance liquid chromatography, was expressed as a percentage and had a normal healthy range of 4.5 - 8%. Therefore, subjects who had baseline HbA1c values > 8% were assigned to the poorly-controlled group (n=20), whereas subjects who presented HbA1c levels ≤ 8% were assigned to the well-controlled group (n=18).

Clinical monitoring

All clinical examinations were performed by one examiner, calibrated according to the method described by Araujo et al.[19] The intra-examiner variability was 0.21 mm for PD and 0.25 mm for CAL. This trained examiner was able to provide reproducible measurements of under 0.5 mm. The clinical parameters registered dichotomously, i.e. bleeding on probing (BoP) and suppuration (SUP), were calculated by the Kappa-Light test and the intra-examiner agreement was > 0.85. The examiner was unaware of the glycemic status of the subjects.

The following parameters were assessed at six sites of all teeth, excluding third molars (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual), using a manual periodontal probe[†] 1- Visible plaque accumulation (PI): presence or absence of plaque along the cervical margin.[20] 2- BoP: presence or absence of bleeding of up to 15 seconds after gentle probing; 3- SUP: presence or absence of spontaneous SUP or SUP on probing; 4- PD (mm): distance between the gingival margin and the bottom of the sulcus/pocket; 5- CAL (mm): distance between cement-enamel junction and the bottom of

[†] UNC15, Hu-Friedy, Chicago, IL, USA

the sulcus/pocket. Clinical examinations were assessed at baseline and 3 and 6 months after periodontal therapy.

GCF sampling

At baseline, GCF was sampled one week after clinical examination so as not to alter the nature of the GCF. Two non-contiguous sites per subject presenting PD and CAL ≥ 5mm, BoP and no furcation involvement were randomly chosen for sampling. After removing the supragingival biofilm with sterile cotton pellets, the sites were isolated with cotton rolls and gently dried with an air syringe to eliminate the possibility of contamination with saliva. GCF was collected by inserting standard paper strips[‡] approximately 2 mm into the sulci/pocket for 30s. Strips visually contaminated with blood were discarded. The GCF sample volume was measured in a calibrated Periotron 8000[§] and the readings were then converted to an actual volume (µl) by reference to the standard curve. The two strips were immediately placed in separate microcentrifuge tubes containing 250 µl phosphate-buffered saline (PBS) plus protease inhibitor cocktail[‖]. The samples were stored at -20ºC for subsequent assays. GCF samples were also taken from the same sites at 3 and 6 months after periodontal therapy. At these times, clinical parameters were recorded and the supportive therapy was performed after GCF sampling.

sRANKL and OPG enzyme linked immunosorbent assay (ELISA)

GCF samples were analyzed by ELISA for sRANKL and OPG using commercially available ELISA kits[¶]. The tubes were vortexed for 30s and centrifuged for 5min at 1,500xg in order to elute. Assays were carried out according to the manufacturer's recommendations

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[‡] Periopaper, Oraflow Inc., Smithtown, NY, USA
[§] Periotron 8000, Proflow Inc., Amityville, NY, USA
[‖] Sigma-Aldrich, Saint Louis, MO, USA
[¶] Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria

using human recombinant standards. 100 µl and 50 µl of each sample were added in each well for RANKL and OPG assays, respectively. The OPG coating antibody was a monoclonal anti-OPG while the OPG detection antibody was a goat polyclonal biotinylated anti-human OPG. For RANKL analysis, the microtiter strips were coated with human recombinant OPG and the RANKL detection antibody was a goat polyclonal biotinylated anti-human sRANKL. The optical density was measured at 450nm. The absorbance readings were converted to amount of RANKL or OPG per well using a trendline equation prepared based on the readings of the standard curve supplied by the manufacturer. The concentrations of the recombinant standard ranged from 0 to 6 pmol/L and from 0 to 30 pmol/L for RANKL and OPG, respectively. The negative controls for both assays were the PBS plus protease inhibitor cocktail without GCF samples. Results are reported as total amount (pg) of sRANKL and OPG per site in 30s. Sites with sRANKL or OPG levels below the detection limit of assay were scored as 0 pg. Calculation of sRANKL and OPG concentration in each GCF sample (pg/µl) was established by dividing the total amount of the each protein by the volume of the fluid.

Periodontal treatment

All subjects were first submitted to the hygiene phase of the periodontal therapy, including supragingival plaque and calculus removal, exodontia, provisional restoration and overhangs of fillings removal. They were instructed to perform a brushing technique using a soft toothbrush, dental floss and interdental toothbrushes, as necessary. Moreover, all volunteers received the same brand of toothpaste[#] to use during the course of the study. Scaling and root planing (SRP) was performed in two to four appointments lasting

[#] Colgate Total®, Anakol Ind. Com. Ltda - Kolynos do Brasil - Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil

approximately 60 minute each under local anesthesia (3% prilocaine with felipressin) using periodontal curettes[**] and ultrasonic device[††]. Treatment was concluded in a maximum of 21 days by the same operator without use of antibiotics or local antimicrobials. At 3 and 6 months post-therapy, all subjects received supportive therapy, including professional plaque control with abrasive sodium carbonate air-powder system[††] and re-instruction of oral hygiene. The subjects were asked to report any changes in the DM treatment regimen in the follow-up appointments.

Sample size calculation

The number of sites for GCF sampling in this study was based on previous studies that found differences in the levels of RANKL and OPG in the GCF when comparing different clinical periodontal status.[18,19] The ideal sample size to assure adequate power for clinical parameter differences was calculated considering differences of at least 0.8 mm for CAL and a standard deviation of 0.94 mm between groups in initially deep periodontal pockets (>6mm). Based on these calculations, it was decided that 17 subjects per group would be necessary to provide an 80% power at significance level of 5%.

Statistical Analysis

The statistical analysis was performed using a commercially available software program[‡‡]. The biostatician was unaware of the glycemic status of the subjects. Data were first examined for normality by the Kolmogorov-Smirnov test and, the data that did not achieve normality was analyzed using non-parametric methods. The study unit for sRANKL and OPG levels was the site rather than the subject since periodontitis is a site-specific disease. The primary variables were differences in the levels of OPG, sRANKL and RANKL/OPG

[**] Hu-Friedy, Chicago, IL, USA
[††] Jet Sonic, Gnatus, Ribeirão Preto, SP, Brazil
[‡‡] SPSS® for Windows version 12.0, SPSS Inc., Chicago, IL, USA

ratio. The secondary variables were clinical parameters, GCF volume and plasma levels of HbA1c and FPG. The percentage of sites with visible plaque, BoP and SUP, the mean PD, CAL, GCF volume and the levels of HbA1c and FPG were computed for each subject and averaged in the glycemic groups. The significance of clinical and glycemic differences between groups was compared using the Mann-Whitney *U*-test. The Student t test was used to compare age, duration of DM, OPG and sRANKL levels and RANKL/OPG ratio between well-controlled and poorly-controlled groups. The Friedman test was employed to detect statistically significant differences within glycemic groups among experimental periods in relation to clinical and glycemic differences. When there were significant differences by the Friedman test, a pair-wise comparison was performed by the Wilcoxon test. Repeated measures ANOVA was employed to detect statistically significant differences in osteoclastogenesis related-factors within glycemic groups among experimental periods. When there were significant differences by the repeated measures ANOVA, a pairwise comparison was performed using Tukey test. The Chi-square test was used to detect differences in the frequencies of gender and treatment regimen between groups. Spearman's Rank Correlation was used to test possible relationships between sRANKL, OPG and RANKL/OPG levels and the clinical parameters of the sampled sites and HbA1c levels. The significance level established for all analyses was 5%.

RESULTS

Retention

There were no subjects and sites dropouts during the course of the study period. Thus, a total of 38 subjects completed the study, 20 poorly-controlled and 18 well-controlled. Seventy-six samples of GCF were analyzed per period, totalizing two hundred and twenty-eight samples. The subjects from both groups reported no adverse effects such as fever and

indisposition after treatment. No changes in the medication and diet were reported by the subjects during the study period.

RANKL and OPG levels

Total amounts (pg/site) and concentrations (pg/µl) of sRANKL and OPG of poorly-controlled and well-controlled subjects at baseline and 3 and 6 months post-therapy are presented in Figures 1 and 2, respectively. Figure 3 presents the ratio of RANKL/OPG in both groups during the experimental period. Total amounts and concentrations of sRANKL and the ratio of RANKL/OPG were significantly higher in poorly-controlled than well-controlled subjects in all experimental periods ($p<0.05$). Total amounts of OPG were higher in well-controlled than poorly-controlled individuals at baseline and 3 months post-therapy ($p<0.05$). There were no statistically significant differences between groups regarding OPG concentration at any time point ($p>0.05$). There was a significant decrease in the total amounts of sRANKL at 3 months post-SRP in the poorly-controlled group ($p<0.05$). In addition, there were increases in sRANKL and OPG concentrations in the poorly-controlled group at 3 months after treatment ($p<0.05$). Well-controlled subjects presented increases in sRANKL concentration at 3 months and in OPG concentration at 3 and 6 months post-therapy ($p<0.05$). In addition, the ratio of RANKL/OPG decreased in well-controlled ($p<0.05$), but not in poorly-controlled subjects at 3 months after treatment ($p>0.05$). At 6 months post-therapy, the RANKL/OPG ratio remained similar to the baseline ratio for both groups.

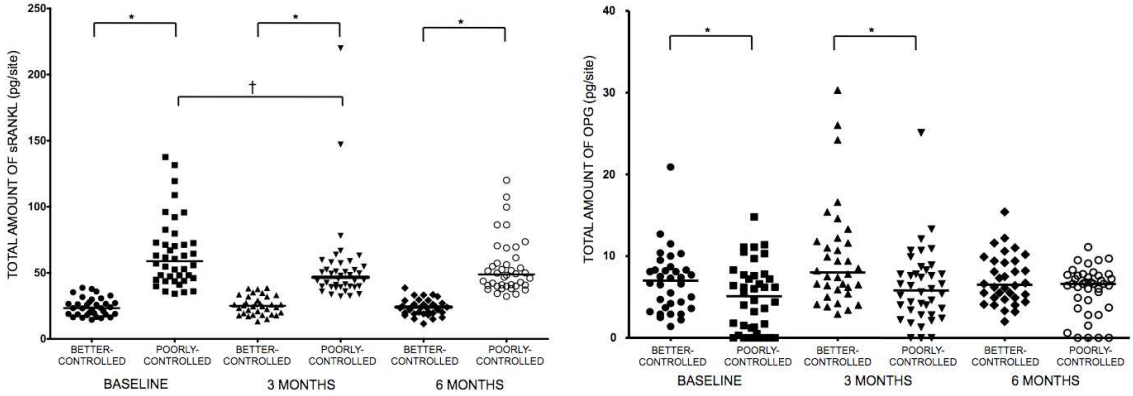

Figure 1. Distribution of the total amounts (pg/site/30s) of sRANKL and OPG in the GCF of poorly- and well-controlled subjects, before and after therapy The horizontal bars show the median values in pg/site. The individual dot represents the total amount at each site. * Differences between groups at each time point (Student t test; $p<0.05$). † Differences over time for each glycemic group (Repeated measures ANOVA and Tukey test; $p<0.05$).

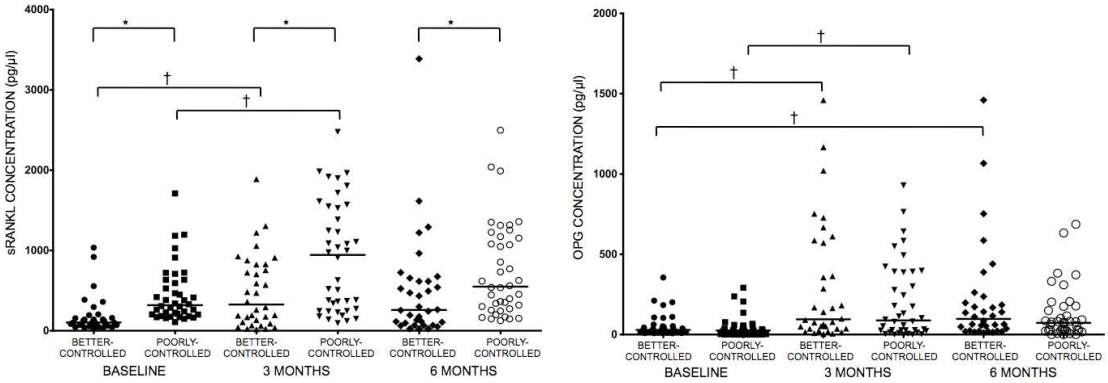

Figure 2. Distribution of the concentration (pg/µl) of sRANKL and OPG in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median value in pg/site. The individual dot represents the concentration at each site. * Differences between groups at each time point (Student t test; $p<0.05$). † Differences over time for each glycemic group (Repeated measures ANOVA and Tukey test; $p<0.05$).

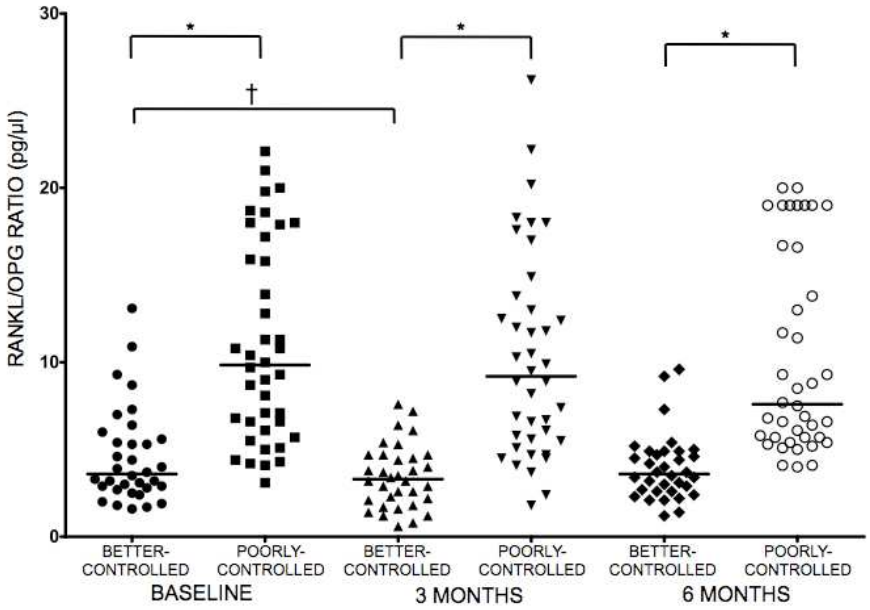

Figure 3. Distribution of the ratio of RANKL/OPG in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median value while the individual dot represents the ratio at each site. * Differences between groups at each time point (Student t test; $p<0.05$). † Differences over time for each glycemic group (Repeated measures ANOVA and Tukey test; $p<0.05$).

Clinical results

Demographic characteristics of the study population at baseline for both groups are presented in Table 1. No significant differences were observed between glycemic groups for age, gender, duration of DM and treatment regimen ($p>0.05$).

The mean (±SD) levels of PD and CAL of the sampled sites were 5.8 ± 1.0 and 5.8 ± 1.0 and, 5.7 ± 1.0 and 6.2 ± 1.6 for well-controlled and poorly-controlled groups, respectively, without significant differences between groups ($p>0.05$).

The full-mouth clinical parameters, HbA1c, FPG and GCF levels of well-controlled and poorly-controlled subjects at baseline and at 3 and 6 months post-therapy are presented in Table 2. Statistically significant decreases in all clinical parameters, except SUP, and in GCF volumes were observed for poorly-controlled and well-controlled subjects at 3 and 6 months post-therapy ($p<0.05$). HbA1c and FPG did not change for both groups over time ($p>0.05$). Diabetic subjects with poor glycemic control had significantly higher visible plaque accumulation (82.0 ± 22.9%) than those with good glycemic control (51.9 ± 23.9%) at baseline ($p<0.05$). Well-controlled subjects achieved a lower mean of CAL (3.1 ± 0.5 mm) than poorly-controlled subjects (3.5 ± 0.8 mm) at 6 months after SRP ($p<0.05$). The mean levels of HbA1c and FPG were higher for poorly-controlled than well-controlled subjects in all experimental periods.

Table 1 - Demographic characteristics of the study population.

Characteristics	Poorly-controlled (n=20)	Well-controlled (n=18)
Age (years)		
Mean ± SD	53.4 ± 8.0	51.2 ± 9.9
Range	42 - 70	40 - 68
Gender (n)		
Male	9	8
Female	11	10
Duration of DM		
Mean ± SD (years)	6.2 ± 0.9	6.1 ± 0.7
Treatment regimen		
Diet	3	4
Diet + insulin	3	2
Diet + oral hypoglycemic agents (metformin or glibenclamin)	12	10
Diet + oral hypoglycemic agents (metformin or glibenclamin) + insulin	2	2

There were no differences between groups regarding age, duration of DM (Student t test; $p>0.05$), gender and treatment regimen (Chi-square test; $p>0.05$).

Table 2 – Full-mouth clinical and, glycemic parameters of poorly-controlled (HbA1c values > 8%) and well-controlled (HbA1c levels ≤ 8%) diabetic subjects before and after scaling and root planing.

Clinical and glycemic parameters	Experimental period	Poorly-controlled (n=20)	Well-controlled (n=18)
PI (%)	Baseline*	82.0 ± 22.9 [a]	51.9 ± 23.9 [a]
	3 months	31.3 ± 21.7 [b]	29.9 ± 24.8 [b]
	6 months	29.9 ± 10.5 [b]	29.7 ± 28.4 [b]
BoP (%)	Baseline	57.2 ± 31.5 [a]	49.9 ± 28.9 [a]
	3 months	8.8 ± 7.5 [b]	12.0 ± 11.2 [b]
	6 months	7.2 ± 7.5 [b]	12.4 ± 14.2 [b]
SUP (%)	Baseline	2.9 ± 4.6 [a]	2.9 ± 5.8 [a]
	3 months	2.0 ± 3.8 [a]	0.6 ± 1.3 [a]
	6 months	0.7 ± 1.8 [a]	0.9 ± 1.3 [a]
PD (mm)	Baseline	3.4 ± 0.6 [a]	3.4 ± 0.8 [a]
	3 months	2.5 ± 0.9 [b]	2.7 ± 0.4 [b]
	6 months	2.7 ± 0.4 [b]	2.5 ± 0.8 [b]
CAL (mm)	Baseline	4.2 ± 0.8 [a]	4.0 ± 0.9 [a]
	3 months	3.5 ± 0.8 [b]	3.2 ± 0.6 [b]
	6 months*	3.5 ± 0.8 [b]	3.1 ± 0.5 [b]
HbA1c (%)	Baseline *	10.5 ± 1.2 [a]	7.0 ± 0.9 [a]
	3 months*	10.1 ± 2.4 [a]	8.7 ± 0.8 [a]
	6 months*	10.9 ± 2.0 [a]	8.3 ± 0.2 [a]
FPG (mg/dl)	Baseline *	196.7 ± 56.7 [a]	129.9 ± 40.8 [a]
	3 months*	209.3 ± 74.4 [a]	147.2 ± 34.9 [a]
	6 months*	224.0 ± 78.8 [a]	151.8 ± 51.0 [a]
GCF volume (µl)	Baseline	0.40 ± 0.15 [a]	0.40 ± 0.17 [a]
	3 months	0.15 ± 0.05 [b]	0.17 ± 0.07 [b]
	6 months	0.15 ± 0.06 [b]	0.15 ± 0.05 [b]

PI: plaque index; BoP: bleeding on probing; SUP: suppuration; PD: probing depth; CAL: clinical attachment level; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; GCF: gingival crevicular fluid. Different letters indicate statistically significant differences over time within each experimental group (Friedman and Wilcoxon tests; $p<0.05$). * Differences between well-controlled and poorly-controlled groups at each time point (Mann-Whitney; $p<0.05$).

Correlations

Table 3 presents the correlation coefficients for total amounts and concentrations of osteoclastogenesis-related factors and the clinical parameters of the sampled sites and HbA1c. Statistically significant positive correlations were found between total amounts and concentration of sRANKL and RANKL/OPG ratio and HbA1c and FPG levels ($p<0.01$). Total amount and concentration of OPG were negatively correlated with HbA1c and FPG levels ($p<0.05$). In addition, the total amount of sRANKL and the RANKL/OPG ratio were positively correlated PI ($p<0.01$).

Table 3 - Correlation coefficients for osteoclastogenesis-related factors and the clinical parameters of the sampled sites and HbA1c levels.

Clinical and glycemic parameters	**Total amount**		**Concentration**		**Ratio**
	sRANKL	**OPG**	**sRANKL**	**OPG**	**RANKL/OPG**
PI	0.420**	-0.124	0.210	0.217	0.345**
BoP	0.116	0.081	0.124	0.104	0.021
SUP	0.052	0.057	0.066	0	-0.078
PD (mm)	-0.025	0.045	0.041	0.100	-0.051
CAL (mm)	0.089	0.049	0.137	0.077	0.034
FPG (mg/dl)	0.571 **	-0.312 *	0.288**	-0.341*	0.514**
HbA1c (%)	0.751 **	-0.284 *	0.393**	-0.291*	0.612**

PI: plaque index; BoP: bleeding on probing; SUP: suppuration; PD: probing depth; CAL: clinical attachment level; HbA1c: glycated hemoglobin. * Correlations significant at $p<0.05$ level by Spearman's Rank Correlation test. ** Correlations significant at the $p<0.01$ level by Spearman's Rank Correlation test.

DISCUSSION

RANKL and OPG are well recognized as important positive and negative regulators, respectively, of osteoclastogenesis and bone resorption. [7,9] This is the first study showing a possible modulation of the RANKL/OPG ratio in sites with periodontitis, according to the glycemic status of subjects with type 2 DM. The results suggest that poor glycemic control may promote an imbalance in the RANKL/OPG system in favor of osteoclastogenesis and, consequently, poorly-controlled individuals may have greater risk of tooth-supporting bone loss than well-controlled subjects.

In vitro studies have demonstrated that periodontal pathogen virulence factors may exert effects on CD4+ T and osteoprogenitor cells regulating the RANKL-OPG system in favor of the osteoclast formation and consequent bone resorption.[21-22] Studies using ELISA showed an increased concentration of RANKL and decreased levels of OPG in the GCF of non-diabetic subjects with periodontitis.[11-14] The hypothesis that an imbalance in RANKL/OPG ratio in periodontal tissues is associated with periodontitis severity was also confirmed by studies using immunohistochemical staining and polymerase chain reaction (PCR)[10,13,23]. In this study, sites with periodontitis from poorly-controlled diabetic subjects presented higher levels of sRANKL and lower levels of OPG in almost all experimental periods, reflecting in a higher RANKL/OPG ratio when compared to well-controlled subjects. In addition, sRANKL levels and the RANKL/OPG ratio in the GCF were positively correlated with the plasma concentration of HbA1c. One could argue that these findings could be attributed to the effect of the severity of disease in sampled sites and not to the glycemic status of the subjects. However, it is important to emphasize that intense effort was made to minimize the impact of disease severity by selecting sites that were clinically similar for PD and BoP between groups. Therefore, together, these data suggest

that hyperglycemia may contribute to a higher RANKL/OPG ratio in periodontal tissues and could, at least in part, be critical for regulating alveolar bone destruction in diabetic subjects with periodontitis. The present molecular results may support clinical findings demonstrating that poorly-controlled subjects present more periodontal destruction than well-controlled diabetic subjects.[4-5]

Some studies have already evaluated the relationship between periodontitis and DM focusing on the role of RANKL and OPG; however, differences in the type of study and DM have hampered a direct comparison with the results of the present study.[16-17,24] Recently, Lappin et al.[17] showed high OPG and low RANKL/OPG ratio in the plasma of type 1 diabetic subjects with periodontitis, presenting low (<8.5%) or high (>8.5%) HbA1c. In addition, in contrast to our results from GCF, the plasma concentration of OPG was positively correlated with HbA1c in blood. The authors attributed these intriguing results to the OPG function in protecting the pancreatic beta cells from further damage in diabetic subjects. In fact, although OPG was firstly described as a bone-related glycoprotein, it may also play other systemic roles in humans, especially those related to the vasculature. One example is that OPG-deficient animals presented a decrease in bone density and presence of vascular calcification.[25]

Several cellular and molecular mechanisms have been proposed to explain the up regulation of RANKL/OPG ratio observed in the GCF of poorly-controlled subjects and the manner by which hyperglycemia may intensify the severity of periodontitis among uncontrolled type 2 DM subjects. One of these involves the non-enzymatic glycation proteins and consequent production and accumulation of advanced glycation end products (AGEs) in tissues.[26] The interaction between AGE and its receptor (RAGE), present in different types of cells, enhances the expression of proinflammatory cytokines, including

interleukin (IL)-1, IL-17, and tumor necrosis factor (TNF)-α.[27-29] A recent study showed that diabetes-enhanced TNF-α increases the expression of resorptive factors in chondrocytes and that TNF-α dysregulation leads to enhanced osteoclast formation and accelerated loss of cartilage in diabetic rats.[30] The pro-inflammatory cytokines increase RANKL and downregulate OPG expressions by various cells.[31-32] In addition, chronic hyperglycemia may modulate bone metabolism by the direct effects of AGE-RAGE interplay on bone cells.[33] RAGE-deficient rats have been shown to demonstrate an increased bone mass and a decreased number of osteoclasts, compared to wild-type rats.[34] Blockage of RAGE decreased alveolar bone loss in rats infected by *Porphyromonas gingivalis*.[29] Franke et al.[35] showed increased RANKL mRNA expression in human osteoblasts exposed to AGEs, indicating that AGE may induce RANKL-mediated osteoclastogenesis. Therefore, it is reasonable to suggest that the upregulation of the RANKL/OPG ratio observed in poorly-controlled subjects of the present study may be a result of the effect of periodontal pathogens and the indirect and direct effects of hyperglycemia on the synthesis of proinflammatory cytokines and on bone cells, respectively.

To data, few interventional studies have assessed separately the effect of periodontal therapy in well-controlled and poorly-controlled diabetic individuals.[36-38] Therefore, the present study also evaluated the effect of the glycemic control on the clinical and glycemic response to SRP. The clinical parameters, except SUP, improved after periodontal therapy for both groups, corroborating previous studies in which SRP was also effective in treating diabetic subjects with periodontitis.[38-40] Interestingly, poorly-controlled subjects presented higher levels of plaque accumulation than well-controlled individuals. This finding is in

accordance to previous investigations showing improved compliance to oral hygiene among diabetic subjects with good glycemic control.[41-42] The mean CAL was lower for the well-controlled than for the poorly-controlled subjects at 6 months, as demonstrated by a previous study from our research group.[38] Differences in sample size, type of DM and experimental design have hampered a comparison between our clinical findings and those from other previous studies that also evaluated the effect of periodontal treatment in diabetic subjects according the glycemic control.[36-37] The levels of HbA1c and FPG did not change significantly for both groups at either 3 or 6 months post-therapy. These findings are in agreement with those from previous investigations in which periodontal therapy resulted in no significant changes in the glycemic control of diabetic subjects.[43-44]

In order to parallel the clinical changes with the osteoclastogenesis-related factors profile in the GCF of poorly and well-controlled subjects, sRANKL and OPG were also assessed at 3 and 6 months post-therapy. It is worthy of note that the diabetic subjects maintained their baseline glycemic status and medication during the study. In addition, DM treatment regimen was not different between poorly and well-controlled individuals. Although it has been demonstrated that anti-diabetic drugs from the thiazolidinedione class may influence RANKL levels and bone resorption[45], none of the subjects included in the present study was under this kind of treatment, reducing a possible influence of DM treatment on the present results. The concentration, but not total amount, of sRANKL and OPG increased for both groups after therapy. However, inconsistencies between concentration and total amount of cytokines in GCF after therapy can be explained by the decrease of GCF volume after treatment due to reduction in the periodontal inflammatory process.[46] Since low levels of RANKL and high levels of OPG have been related to healthy periodontal tissues[11-14], it would be expected that significant improvements in the RANKL/OPG ratio

should accompany clinical improvements post-therapy. However, although there was a trend towards the reduction of RANKL/OPG ratio in both groups, it was only significant in the well-controlled subjects at 3 months after SRP. Therefore, it could be speculated that the worse clinical results (i.e. higher CAL level) observed for poorly-controlled subjects at 6 months may be a consequence of the persistent hyperglycemia after treatment that may produce a remaining inflammatory process and a continual imbalance in RANKL/OPG ratio in periodontal tissues. Further evaluations are necessary to clarify this issue since a network of inflammatory mediators are involved in the pathogenesis of periodontal diseases. Taken together, these findings reinforce the hypothesis that chronic hyperglycemia (longer duration and poor metabolic control) may negatively influence the post-therapy RANKL/OPG ratio in the GCF of sites of periodontitis in type 2 diabetic subjects.

In conclusion, RANKL/OPG ratio in untreated and treated periodontitis sites may be influenced by a poor glycemic control in type 2 diabetic subjects. The consequence of these findings is that periodontal bone resorption may be mediated by the glycemic status of these subjects. Studies on the molecular mechanisms of bone destruction in periodontitis may be useful to suggest the use of bone-resorption modulation agents as adjunctive therapy to anti-infectious strategies[47], especially in groups at risk from periodontal breakdown, such as poorly-controlled diabetic subjects.

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4 . ESTUDO 2

Cytokine levels in sites of chronic periodontitis of poorly-controlled and well-controlled type 2 diabetic subjects - J Clin Periodontol. 2010 Dec;37(12):1049-58.

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Abstract

Aim: This study compared the levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-4, IL-17 and IL-23 in the gingival crevicular fluid (GCF) from well-controlled and poorly-controlled type 2 diabetic subjects with chronic periodontitis, before and after periodontal therapy.

Methods: Eighteen well-controlled (glycated hemoglobin levels ≤ 8%) and 20 poorly-controlled (glycated hemoglobin levels > 8%) diabetic subjects were enrolled in this study. All subjects were submitted to non-surgical periodontal therapy. GCF sampling and clinical periodontal parameters were assessed before, 3 and 6 months post-therapy. Total amounts and concentrations of TNF-α, IFN-γ, IL-4, IL-17 and IL-23 in the GCF were analyzed by ELISA.

Results: The levels of IL-17 were higher in poorly- than in well-controlled subjects ($p<0.05$), whereas the levels of IFN-γ were increased in well- compared to poorly-controlled subjects at all experimental groups ($p<0.05$). In addition, IL-4 levels were lower in well- than poorly-controlled diabetic subjects at baseline ($p<0.05$). There were no differences between groups for TNF-α and IL-23 at any time points ($p>0.05$).

Conclusion: These results indicate a predominance of pro-inflammatory Th1- or Th17-cytokines in sites of chronic periodontitis from type 2 diabetic subjects, according to their glycemic control.

Key-words: diabetes mellitus; chronic periodontitis; root planing; inflammation; cytokines; Enzyme-Linked Immunosorbent Assay.

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Scientific rationale for study: Evidence indicates that the host response to periodontal infection in diabetic subjects may be influenced by glycemia. It is important to clarify these mechanisms and determine the Th-derived cytokine profile in periodontitis of diabetic subjects, according to their glycemic condition. **Principal findings:** Periodontitis in well-controlled type 2 diabetic subjects presented a predominance of a Th1-type cytokine (IFN-γ), while in poorly-controlled individuals exhibited dominance of Th17-type cytokine (IL-17). **Practical implications:** The understanding of host immunoinflammatory response may be useful to suggest modulation agents as adjunctive to anti-infectious therapies, especially in groups at risk, such as diabetic subjects.

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Introduction

Type 2 diabetes mellitus (DM), characterized by impaired insulin function due to changes in insulin molecules and/or their cell receptors (Kidambi & Patel 2008), is the most prevalent type of DM among middle-aged subjects (Israili 2009). Several studies have demonstrated that the prevalence, progression and severity of periodontal diseases are higher in diabetic subjects when compared to non-diabetic ones, supporting DM as a risk factor for periodontitis (Kinane & Bouchard 2008). In addition, clinical studies have

demonstrated a positive association between poor glycemic control and the severity of periodontal diseases (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Chen et al. 2010, Bandyopadhyay et al. 2010).

Periodontitis is an infectious-inflammatory disease that results from the interaction between biofilm and the host defense mechanisms and is sustained by a network of pro- and anti-inflammatory mediators that may play antagonist and/or synergic biological activities (Tatakis & Kuman 2005). After antigenic stimulation, naïve CD4+ T cells, one of the most important cell types in cell-mediated immune response, may differentiate into effector T helper (Th) cells including Th1, Th2 and Th17 phenotypes, each with distinct profiles of cytokine production (Tesmer et al. 2008, Zhu et al. 2010). Interferon (IFN)-γ, the main cytokine secreted by Th1 subsets and natural killer cells, induces the macrophage activation and the production of a series of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α. Increased numbers of IFN-γ-producing cells in periodontal tissues and elevated levels of IFN-γ in the gingival crevicular fluid (GCF) have been associated with the progression of chronic periodontitis (Ukai et al. 2001, Dutzan et al. 2009b). Conversely, interleukin (IL)-4, an anti-inflammatory cytokine secreted by Th2 cells, downregulates the production of IFN-γ and other pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β (Mosmann & Coffman 1989, te Velde et al. 1990) and, therefore, its presence has been related to healthy periodontal tissues (Shapira et al. 1992, Kabashima et al. 1996, Tsai et al. 2007). IL-17 is a pro-inflammatory cytokine, produced by Th17 subsets, which promotes recruitment of neutrophils (Tesmer et al. 2008) and stimulates the production of a series of pro-inflammatory mediators (Patel et al. 2007, Tesmer et al. 2008), matrix metalloproteinases (MMP) and osteoclastogenesis related-factors (Oda et al.

2003, Takahashi et al. 2005, Sato et al. 2006, Beklen et al. 2007). Elevated levels of IL-17 messenger RNA (mRNA) and protein, as well as the presence of Th17 cells, have been observed in diseased, when compared to healthy periodontal tissues (Takahashi et al. 2005, Cardoso et al. 2009). IL-23 is essential in maintaining and expanding the Th17 cell population and plays a critical role in driving an initial inflammatory immune response against pathogens or injuries by inducing IL-17 production and neutrophil recruitment (Tan et al. 2009). Increased levels of IL-23 in the gingival tissue have been observed in sites presenting clinical attachment loss (Lester et al. 2007).

Although the clinical relationship between periodontitis and DM is well established, few investigations have focused on the immunoinflammatory responses in sites with periodontitis in subjects with DM (Engebretson et al. 2004, Engebretson et al. 2006, Duarte et al. 2007, Navarro-Sanchez et al. 2007, Venza et al. 2010). In addition, the cellular and molecular mechanisms that could explain the more severe clinical periodontal destruction observed in poorly-controlled diabetic subjects, when compared to well-controlled subjects, are still unclear (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Chen et al. 2010, Bandyopadhyay et al. 2010). Therefore, the aim of this study was to compare the levels of TNF-α, IFN-γ, IL-4, IL-17 and IL-23 in the GCF from well-controlled and poorly-controlled type 2 diabetic subjects with chronic periodontitis before and after non-surgical periodontal therapy. The hypothesis is that glycemic control may alter the protective and destructive host immune and inflammatory responses to periodontal pathogens in type 2 diabetic subjects.

Material and Methods

Subject population

Thirty-eight subjects (age range: 40 to 67 years) diagnosed with type 2 DM and chronic periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University, from July 2007 until March 2008. Detailed medical and dental records were obtained. Subjects who fulfilled the following inclusion/exclusion criteria were invited to participate in the study. All eligible subjects were thoroughly informed of the nature, potential risks and benefits of their participation in the study and signed their informed consent. This study protocol was previously approved by Guarulhos University's Ethics Committee in Clinical Research.

Inclusion and exclusion criteria

Data concerning the duration of DM and medications were retrieved from the medical records of the subjects at the beginning of the study. All subjects had presented diagnosis of type 2 DM during at least the past 5 years and were under insulin supplementation, diet regime and/or oral hypoglycemic agents. Subjects were diagnosed with generalized chronic periodontitis, based on the clinical and radiographic criteria proposed by the 1999 World Workshop for Classification of Periodontal Diseases and Conditions (Armitage 1999). All subjects were > 30 years old, had at least 15 teeth excluding third molars and teeth with advanced decay indicated to exodontias, and more than 30% of the sites had probing depth (PD) and clinical attachment level (CAL) ≥ 5 mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking, smoking within the past 5 years, periodontal or/and antibiotic therapies during the previous 6 months, use of mouth rinses containing antimicrobials in the preceding 2 months, any systemic condition (except DM) that could affect the progression of periodontal disease (e.g. immunological disorders, osteoporosis) and long-term administration of anti-inflammatory and immunosuppressive

medications. Subjects with periapical pathology, orthodontic appliances, and multiple systemic complications of DM were also excluded from the study.

Blood analyses and experimental groups

A single laboratory (Guarulhos University Clinical Analysis Laboratory) performed all blood analyses. Blood samples were taken for each subject at baseline, 3 and 6 months post-therapy. The fasting plasma glucose (FPG), measured using the glucose oxidase method, was expressed in milligrams per deciliter (mg/dl). Glycosylated hemoglobin levels (HbA1c), measured by high-performance liquid chromatography, were expressed as a percentage. Subjects who had baseline HbA1c values > 8% were assigned to the poorly-controlled group (n=20), whereas subjects who presented HbA1c levels ≤ 8% were assigned to the well-controlled group (n=18).

Clinical monitoring

All clinical examinations were performed by one examiner (V.R.S), calibrated according to the method described by Araujo et al. (2003). The intra-examiner variability was 0.21 mm for probing depth (PD) and 0.25 mm for clinical attachment loss (CAL). This trained examiner was able to provide reproducible measurements of under 0.5 mm. The clinical parameters registered dichotomously, i.e. bleeding on probing (BoP) and suppuration (SUP), were calculated by the Kappa-Light test and the intra-examiner agreement was > 0.85. The examiner was unaware of the glycemic status of the subjects.

The following parameters were assessed at six sites of all teeth, excluding third molars (mesio-buccal, medio-buccal, disto-buccal, mesio-lingual, medio-lingual, disto-lingual), using a manual periodontal probe (UNC15, Hu-Friedy, Chicago, IL, USA): visible plaque accumulation (PI) (Ainamo & Bay 1975), BoP, SUP, PD (mm) and CAL (mm). Clinical examinations were assessed at baseline, 3 and 6 months after periodontal therapy.

GCF sampling

At baseline, GCF was sampled one week after clinical examination so as not to alter the nature of the GCF. Two non-contiguous sites per subject presenting PD and CAL ≥ 5mm, BoP and no furcation involvement were chosen for sampling. After removal of the supragingival biofilm with sterile cotton pellets, the sites were isolated with cotton rolls and gently dried with an air syringe to eliminate the possibility of contamination with saliva. GCF was collected by inserting standard paper strips (Periopaper, Oraflow Inc., Smithtown, NY, USA) approximately 2 mm into the sulcus/pocket for 30s. Strips visually contaminated with blood were discarded. The GCF sample volume was measured in a calibrated Periotron 8000 (Periotron 8000, Proflow Inc., Amityville, NY, USA) and the readings were then converted to an actual volume (µl) by reference to the standard curve. The strips from the two selected sites were immediately placed into separate microcentrifuge tubes containing 250 µl phosphate-buffered saline and protease inhibitor cocktail (Sigma-Aldrich, Saint Louis, Missouri, USA). The samples were stored at -20ºC for subsequent assays. GCF samples were also taken from the same sites at 3 and 6 months after periodontal therapy. At these times, clinical parameter measurements and supportive therapy were performed after GCF sampling.

Cytokine enzyme linked immunosorbent assay (ELISA)

GCF samples were analyzed by ELISA for TNF-α, IFN-γ, IL-4, IL-17 and IL-23 using commercially available ELISA kits (Quantikine; R&D Systems Inc., MN, USA). The tubes were vortexed for 30s and centrifuged for 5min at 1,500xg in order to elute. Assays were carried out according to the manufacturer's recommendations using human recombinant standards. The minimum detectable doses (sensitivity) for TNF-α, IL-4 and IL-23 assays range from 0.038 - 0,191 pg/ml, 0.03 - 0.22 pg/ml and 2.7 - 16.3 pg/ml, respectively. The

minimum detectable doses for IFN-γ and IL-17 are less than 8.0 pg/ml and less than 15 pg/ml, respectively. The optical density was measured at 450 or 490 nm, according to each cytokine recommendation. Results are reported as total amount (pg) of each cytokine per site. Sites with cytokine levels below the detection limit of the assay were scored as 0 pg. Calculation of cytokine concentrations in each GCF sample (pg/µl) was established by dividing the total amount of each cytokine by the total volume of the fluid in the site.

Periodontal treatment

Subjects first received supragingival plaque and calculus removal, exodontia, provisional restoration and overhangs of fillings removal. They were instructed to perform a brushing technique using a soft toothbrush, dental floss and interdental toothbrushes, as necessary. Moreover, all volunteers received the same brand of toothpaste to use during the course of the study (Colgate Total®, Anakol Ind. Com. Ltda – Kolynos do Brasil – Colgate Palmolive Co., São Bernardo do Campo, SP, Brazil). Scaling and root planing (SRP) was performed during two to four appointments, lasting approximately 60 minute each, under local anesthesia (3% prilocaine with felypressin) using periodontal curettes (Hu-Friedy, Chicago, IL, USA) and an ultrasonic device (Jet Sonic, Gnatus, Ribeirão Preto, SP, Brazil). Treatment was concluded in a maximum of 21 days by the same operator without use of antibiotics or local antimicrobials. All subjects received supportive therapy, including professional plaque control with an abrasive sodium carbonate air-powder system (Jet Sonic, Gnatus, Ribeirão Preto, SP, Brazil) and re-instruction of oral hygiene, at 3 and 6 months post-therapy. The subjects were asked to report any changes in the DM treatment regimen in the follow-up appointments.

Sample size calculation

The number of sites for GCF sampling in this study was based on previous studies that found differences in the levels of cytokines in the GCF, when comparing different clinical periodontal status (Vernal et al. 2005, Tsai et al. 2007). The ideal sample size to assure adequate power for clinical parameter differences was calculated considering differences of at least 0.8 mm for CAL and a standard deviation of 0.94 mm between groups in initially deep periodontal pockets (>6mm). Based on these calculations, it was decided that 17 subjects per group would be necessary to provide an 80% power at significance level of 5%.

Statistical Analysis

The statistical analysis was performed using a software program (BioEstat 5.0, Sociedade Civil Mamirauá, CNPq, Tefé, AM, Brazil). The biostatistician was unaware of the glycemic status of the subjects. Data were first examined for normality by the Kolmogorov-Smirnov test and the data that did not achieve normality was analyzed using non-parametric methods. The study unit for cytokine levels was the site rather than the subject since periodontitis is a site-specific disease. The primary variables were differences and changes in the levels of each evaluated cytokine. The secondary variables were clinical parameters, GCF volume and plasma levels of HbA1c and FPG. The percentage of sites with visible plaque accumulation, BoP and SUP, the mean PD, CAL, GCF volume and the levels of HbA1c and FPG were computed for each subject. Clinical parameters were averaged across subjects. Subsequently, all data were averaged in the glycemic groups. The significance of clinical and glycemic differences between groups was compared using the Mann-Whitney *U*-test. The Student t test was used to compare age, duration of DM, and cytokine levels between well-controlled and poorly-controlled groups. The Friedman test was employed to detect statistically significant differences within glycemic groups

among experimental periods in relation to clinical and glycemic differences. When there were significant differences by the Friedman test, a pair-wise comparison was performed by the Wilcoxon test. Repeated measures ANOVA was employed to detect statistically significant differences in cytokines within glycemic groups among experimental periods. When there were significant differences by the repeated measures ANOVA, a pair-wise comparison was performed using Tukey test. The Chi-square test was used to detect differences in the frequencies of gender between groups. Spearman's Rank Correlation was used to test possible relationships between cytokine levels and PD and CAL in the same sampled sites. The cytokine levels were also correlated to HbA1c levels by Spearman's Rank Correlation. The significance level established for all analyses was 5%.

Results

Retention

There were no subject and sampling site dropouts during the course of the study period. Thus, a total of 38 subjects completed the study, 20 poorly-controlled and 18 well-controlled. Seventy-six samples of GCF were analyzed per period, totalizing two hundred and twenty-eight samples. The subjects from both groups reported no adverse effects such as fever and indisposition after treatment. No changes in the medication and diet were reported by the subjects during the study period.

Clinical results

No significant differences were observed between glycemic groups for age, gender and duration of DM ($p<0.05$) (Table 1). The mean (±SD) levels of PD and CAL of the sampled sites were 5.8 ± 1.0 and 5.8 ± 1.0 and, 5.7 ± 1.0 and 6.2 ± 1.6 for well-controlled and poorly-controlled groups, respectively. At 3 months post-therapy, PD and CAL of the sampled sites changed for 3.4 ± 0.8 and 5.2 ± 0.4 and, 3.7 ± 0.9 and 5.5 ± 1.0 for well-

controlled and poorly-controlled groups, respectively ($p<0.05$). At 6 months post-therapy, PD and CAL of the sampled sites changed for 3.3 ± 0.6 and 5.2 ± 0.4 and, 3.5 ± 0.8 and 5.6 ± 0.8 for well-controlled and poorly-controlled groups, respectively ($p<0.05$).

Table 1 - Demographic characteristics of the study population.

Characteristics	Poorly-controlled (n=20)	Well-controlled (n=18)
Age (years)		
Mean ± SD	52.33 ± 7.0	52.2 ± 9.7
Range	41 - 66	40 - 67
Gender (n)		
Male	9	8
Female	11	10
Duration of DM		
Mean ± SD (years)	6.2 ± 0.8	6.1 ± 0.6

There were no differences between groups regarding age, duration of DM (Student t test; $p>0.05$) and gender (Chi-square test; $p>0.05$).

Statistically significant decreases in all full-mouth clinical parameters, except SUP, and in GCF volumes were observed for poorly-controlled and well-controlled subjects at 3 and 6 months post-therapy (Table 2, $p<0.05$). The mean levels of HbA1c and FPG did not change for either group over time ($p>0.05$) and remained higher for poorly- than for well-controlled subjects in all experimental periods ($p<0.05$). Diabetic subjects with poor glycemic control had significantly higher visible plaque accumulation than those with good glycemic control at baseline ($p<0.05$). Well-controlled subjects achieved a lower mean of CAL than poorly-controlled subjects at 6 months post-therapy ($p<0.05$).

Table 2 – Full-mouth clinical parameters and glycemic status of poorly-controlled (HbA1c values > 8%) and well-controlled (HbA1c levels ≤ 8%) diabetic subjects before and after scaling and root planing.

	Poorly-controlled (n=20)			**Well-controlled (n=18)**		
Parameters	**Baseline**	**3 months**	**6 months**	**Baseline**	**3 months**	**6 months**
PI (%)	81.3 ± 21.4 [a]*	30.3 ± 20.5 [b]	29.0 ± 9.5 [b]	52.9 ± 22.3 [a]	21.3 ± 24.8 [b]	27.6 ± 22.5 [b]
BoP (%)	53.2 ± 30.5 [a]	7.8 ± 7.3 [b]	7.0 ± 7.3 [b]	49.9 ± 27.8 [a]	11.5 ± 10.2 [b]	13.2 ± 10.4 [b]
SUP (%)	3.0 ± 3.6	2.0 ± 3.6	0.7 ± 1.7	2.8 ± 5.7	1.0 ± 1.3	1.9 ± 1.3
PD (mm)	3.4 ± 0.4 [a]	2.5 ± 0.9 [b]	2.7 ± 0.4 [b]	3.4 ± 0.8 [a]	2.7 ± 0.4 [b]	2.5 ± 0.8 [b]
CAL (mm)	4.2 ± 0.6 [a]	3.5 ± 0.8 [b]	3.6 ± 0.8 [b]*	4.0 ± 0.9 [a]	3.2 ± 0.6 [b]	3.1 ± 0.5 [b]
HbA1c (%)	10.4 ± 1.1*	10.1 ± 2.2*	10.9 ± 2.0*	7.0 ± 0.9	8.3 ± 0.8	8.3 ± 0.2
FPG (mg/dl)	194.6 ± 56.7*	206.3 ± 72.5*	212.0 ± 79.0*	131.9 ± 38.8	145.2 ± 33.9	149.8 ± 51.0
GCF (μl)	0.40 ± 0.15 [a]	0.15 ± 0.05 [b]	0.15 ± 0.06 [b]	0.40 ± 0.16 [a]	0.17 ± 0.07 [b]	0.15 ± 0.05 [b]

PI: plaque index; BoP: bleeding on probing; SUP: suppuration; PD: probing depth; CAL: clinical attachment level; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose; GCF: gingival crevicular fluid.

Different letters indicate statistically significant differences over time within each glycemic group (Friedman and Wilcoxon tests; $p<0.05$). * Differences between well-controlled and poorly-controlled groups at each time point (Mann-Whitney; $p<0.05$).

Cytokine levels

In general, the cytokine levels for both diabetic groups did not change following periodontal therapy ($p>0.05$), except for the concentration of IFN-γ, which increased in well-controlled subjects at 3 and 6 months post-therapy (Figure 1; $p<0.05$). In addition, total amounts and concentrations of IFN-γ were higher in well- than in poorly-controlled

subjects at baseline and 3 and 6 months post-therapy (Figure 1; $p<0.05$). The levels of IL-4 were lower in well- than poorly-controlled diabetic subjects at baseline ($p<0.05$; Figure 2). Conversely, total amount and concentration of IL-17 were higher in poorly- than well-controlled subjects in all experimental periods (Figure 3; $p<0.05$). There were no significant differences between groups, regarding the total amount and concentration of TNF-α (Figure 4) and IL-23 (Figure 5) at any time point ($p>0.05$).

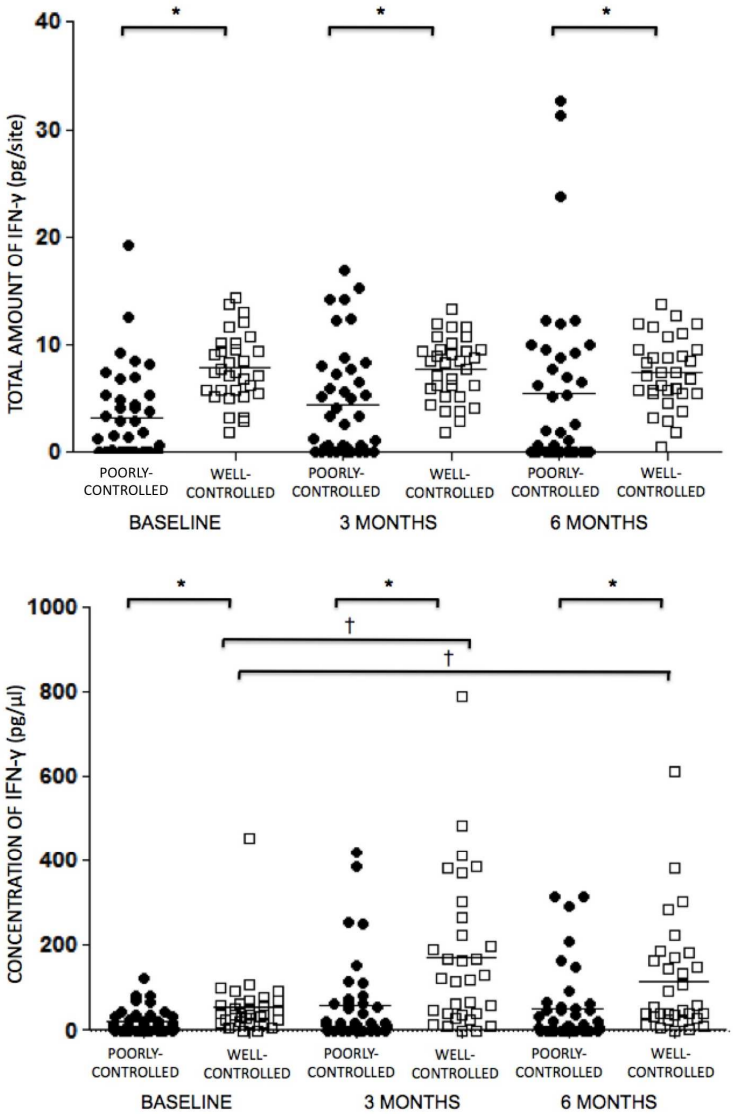

Figure 1. Distribution of the total amount (pg/site) and concentration (pg/µl) of IFN-γ in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. * Differences between groups at each time point (Student t test; $p<0.05$). † Differences over time for each glycemic group (Repeated measures ANOVA and Tukey test; $p<0.05$).

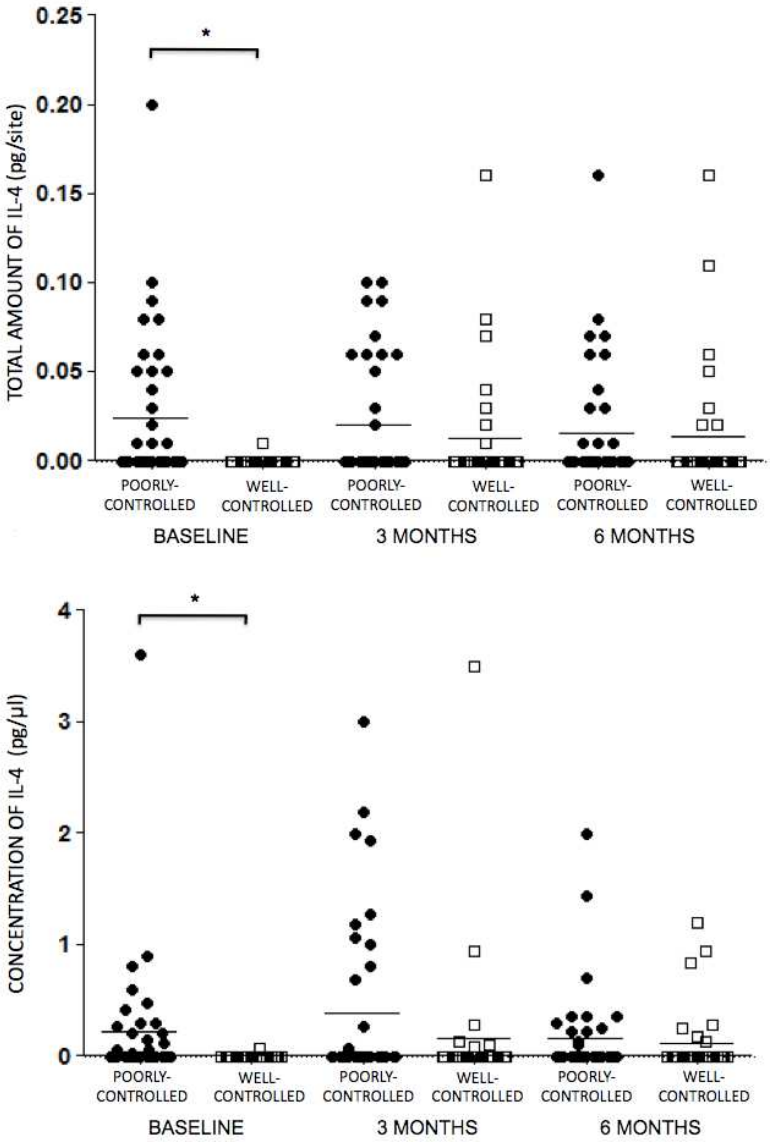

Figure 2. Distribution of the total amount (pg/site) and concentration (pg/µl) of IL-4 in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. * Differences between groups at each time point (Student t test; $p<0.05$).

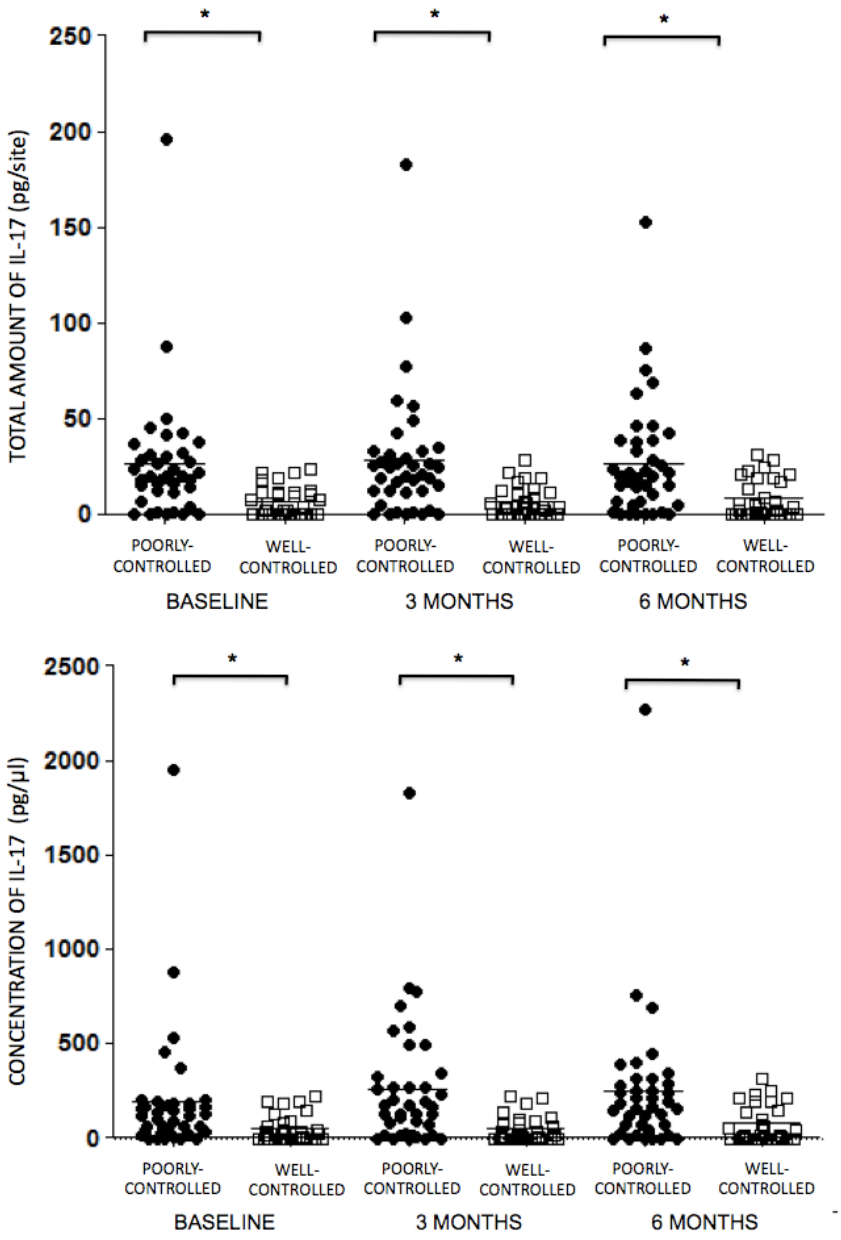

Figure 3. Distribution of the total amount (pg/site) and concentration (pg/µl) of IL-17 in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site. * Differences between groups at each time point (Student t test; $p<0.05$).

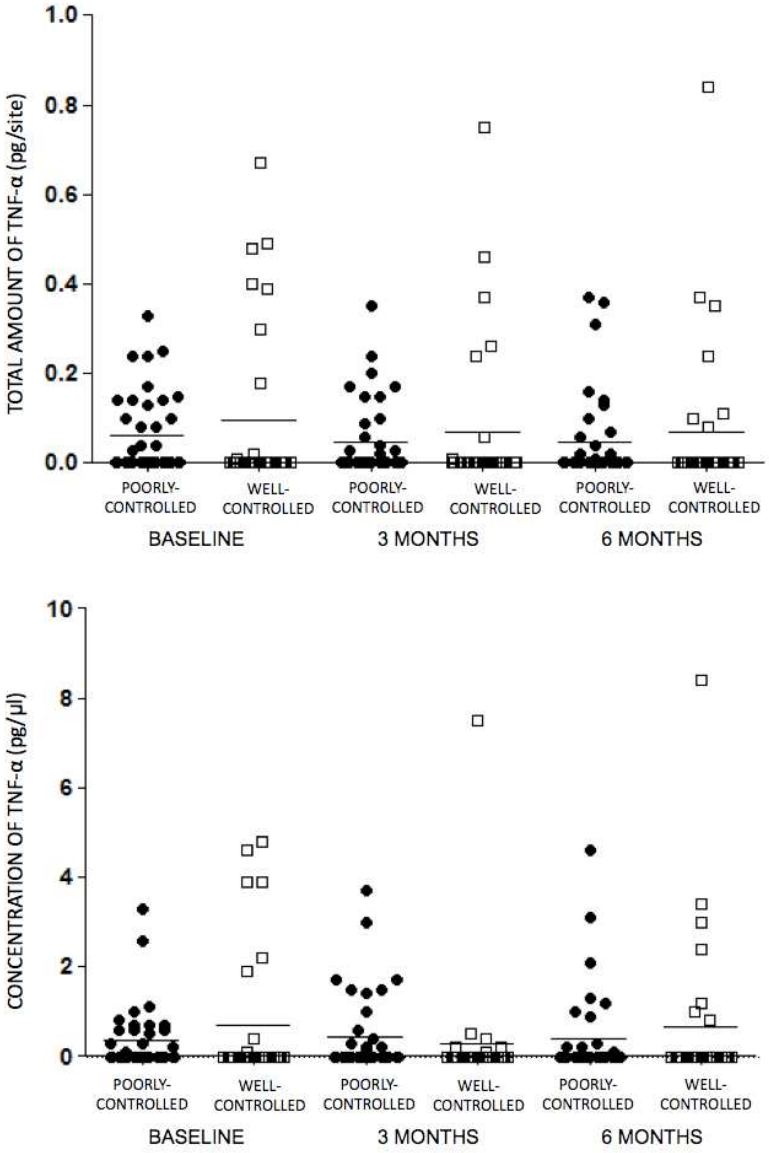

Figure 4. Distribution of the total amount (pg/site) and concentration (pg/µl) of TNF-α in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site.

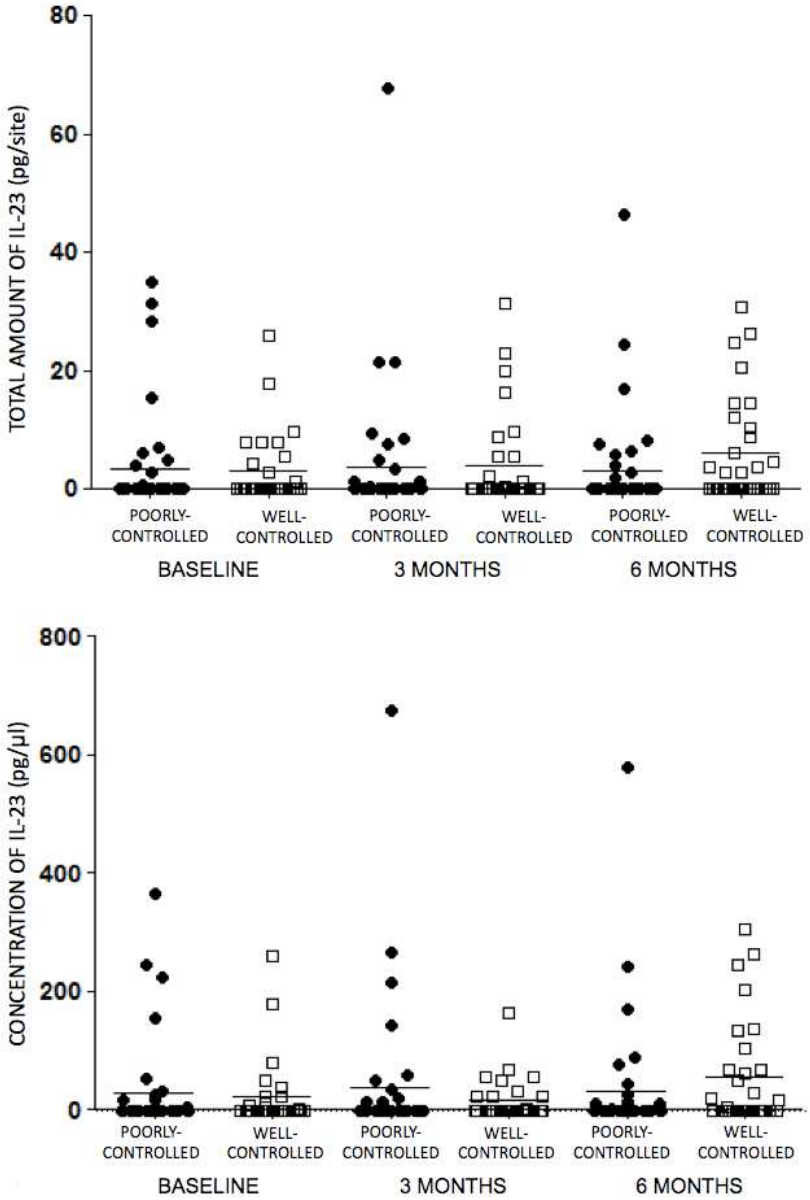

Figure 5. Distribution of the total amount (pg/site) and concentration (pg/µl) of IL-23 in the GCF of poorly- and well-controlled subjects, before and after therapy. The horizontal bars show the median values. The individual dot represents the cytokine level at each site.

Correlations

Table 3 presents the correlation coefficients for total amounts and concentrations of cytokines and PD and CAL of the sampled sites and HbA1c level. Statistically significant positive correlations were found between total amounts and concentration of TNF-α ($p<0.05$), IL-4 ($p<0.001$) and IL-17 ($p<0.001$) and HbA1c while the levels of IFN-γ were negatively correlated with HbA1c ($p<0.001$). In addition, IL-17 was positively correlated with IL-4 ($p<0.001$) and TNF-α ($p<0.05$). There was a negative correlation between the concentration of IFN-γ and the total amount of IL-17.

Table 3 - Correlation coefficients for cytokine levels and PD and CAL of the sampled sites and, HbA1c levels (n= 76 periodontal sites).

		TNF-α		**IFN-γ**		**IL-4**		**IL-17**		**IL-23**	
		TA	CC	TA	CC	TA	CC	TA	CC	TA	CC
TNF-α	CC	**0.98****									
IFN-γ	TA	-0.12									
	CC	-0.25	-0.26	**0.99****							
IL-4	TA	0.03	0.67	-0.20	-0.10						
	CC	0.04	0.08	-0.20	-0.11	**0.99****					
IL-17	TA	**0.26***	**0.30***	-0.20	**-0.27***	**0.51****	**0.51****				
	CC	0.20	**0.25***	-0.08	-0.16	**0.46****	**0.46****	**0.94****			
IL-23	TA	0.15	0.16	0.20	0.04	0.23	0.22	0.20	0.20		
	CC	0.15	0.16	0.18	0.03	0.22	0.22	0.19	0.19	**0.99****	
HbA1c		**0.25***	**0.27***	**-0.60****	**-0.57****	**0.41****	**0.41****	**0.50****	**0.41****	-0.01	-0.01
PD		-0.13	-0.11	0.12	0.18	0.003	0.002	-0.10	-0.10	-0.03	-0.02
CAL		-0.04	-0.09	-0.10	0.20	-0.08	-0.08	-0.07	-0.05	-0.02	-0.01

TA: total amount (pg/site); CC: concentration (pg/µl); HbA1c: glycated hemoglobin (%); PD: probing depth (mm); CAL: clinical attachment level (mm); TNF-α: tumor necrosis factor-α; (IFN)-γ: interferon-γ; IL-4: interleukin-4; IL-17: interleukin-17; IL-23: interleukin-23.

* Correlations significant at $p<0.05$ level by Spearman's Rank Correlation test.

** Correlations significant at the $p<0.001$ level by Spearman's Rank Correlation test.

Discussion

It has been suggested that an imbalance among Th1-, Th2-, Th17- and T regulatory (Treg)-type cytokines in the immune-inflammatory response against periodontal pathogens is critical in the determination of the pattern of periodontal lesions (Seymour & Gemmel 2001, Ukai et al. 2001, Teng 2002, Garlet et al. 2003). This study examined, for the first time, the levels of pro- and anti-inflammatory cytokines related to the Th cells (TNF-α, IFN-γ, IL-4, IL-17 and IL-23) in chronic periodontitis sites of type 2 diabetic subjects presenting good or poor glycemic control. In general, the results demonstrated that the pattern of cytokines in periodontitis sites, matched to disease severity, might be related to the glycemic status, which, in turn, seems to play a crucial role in the host response against periodontal pathogens in diabetic subjects. Sites with chronic periodontitis of well-controlled type 2 diabetic subjects exhibited higher GCF levels of IFN-γ and decreased levels of IL-4, suggesting predominance of Th1-type cytokine in these subjects, while sites of poorly-controlled individuals presented increased IL-17 levels, suggesting dominance of Th17-type cytokines in sites under the challenge of hyperglycemia.

For many years, periodontitis was described as an imbalance between Th1 and Th2 cytokine profiles (Gemmell & Seymour 2004). Recently, this concept has been defied by the discovery of Th17 and Treg cells and their related cytokines in periodontal lesions (Takahashi et al. 2005, Lester et al. 2007, Cardoso et al. 2008, Cardoso et al. 2009, Dutzan et al. 2009a). Overall, clinical and *in vitro* evidence shows presence of the Th17-type immune response in periodontal diseases and indicates that periodontal pathogens can stimulate IL-17 production from T-cells (Oda et al. 2003, Lester et al. 2007, Cardoso et al. 2009). The role of Th17 cells and their related factors in autoimmune type 1 DM has been

often investigated (Bradshaw et al. 2009). However, to date, there is little information regarding the function of these cells in type 2 DM (Arababadi et al. 2010). A recent study showed that the serum levels of IL-17 were higher in type 2 diabetic subjects, when compared to non-diabetic controls, suggesting a relationship between IL-17 and type 2 DM (Arababadi et al. 2010). The precise biological mechanisms that could explain the elevated levels of IL-17 in the GCF of poorly-controlled subjects and the positive correlation between this cytokine and HbA1c levels remain to be truly evaluated. One hypothesis may be the non-enzymatic glycation of proteins under a hyperglycemic condition and, subsequent accumulation of advanced glycation end products (AGEs) in periodontal tissues (Katz et al. 2005). In addition to the stimulation of proinflammatory mediators by pathogens in periodontal sites, AGEs, when attached to its receptors (RAGE), also stimulate the overproduction of proinflammatory cytokines, including IL-17 (King 2008). Accordingly, the destructive biological functions of IL-17 may amplify the severity of periodontal inflammation (Takahashi et al. 2005, Beklen et al. 2007) and link T-cell activation to bone resorption (Sato et al. 2006). In fact, it has been suggested that the Th17-type response may induce osteoclastogenesis, rather than Th1, possibly through IL-17-mediated induction of the receptor activator of NF-$_{\kappa}$B ligand (RANKL) (Kotake et al. 1999, Sato et al. 2006, Dutzan et al. 2009a). Interestingly, recent findings from our research group have shown that poor glycemic control is related to an imbalance in the RANKL/OPG ratio in the GCF from type 2 diabetic subjects, favoring osteoclastogenesis (Santos et al. 2010). Together, these findings reinforce the hypothesis that chronic hyperglycemia may interfere in the levels of specific immune-inflammatory mediators in periodontal tissues that could explain, at least in part, the more severe periodontal breakdown reported in poorly-controlled, when compared to well-controlled diabetic

subjects (Seppälä & Ainamo 1994, Tsai et al. 2002, Lim et al. 2007, Chen et al. 2010, Bandyopadhyay et al. 2010).

Elevated concentrations of IL-23 in diseased periodontal sites, compared to healthy tissues, suggest the involvement of this cytokine in the pathogenesis of periodontitis (Lester et al. 2007, Cardoso et al. 2009, Ohyama et al. 2009). However, to our knowledge, GCF levels of IL-23 have not previously been reported in periodontitis subjects with type 2 DM. Although IL-23 is able to differentiate and expand memory T cells and increase the production of IL-17 (Tan et al. 2009), in this study, no significant differences in the levels of IL-23 were found between groups at any time. It is important to note that, besides IL-23, other cytokines have been suggested to have a role in stabilizing the IL-17-producing T cells, supporting other pathways, differently from IL-23, in the induction of these types of response (Sutton et al. 2006).

IFN-γ and IL-4 correspond to Th1 and Th2 cytokines, respectively. In non-diabetic subjects, it has been demonstrated that low amounts of IL-4 and high levels of IFN-γ are involved in the destruction of periodontal tissues, whereas an increased ratio of IL-4/IFN-γ is associated with healthy periodontal conditions (Shapira et al. 1992, Ukai et al. 2001, Tsai et al. 2007). In this study, periodontitis sites from well-controlled subjects exhibited higher levels of IFN-γ in all experimental periods and decreased levels of IL-4 at baseline, when compared to poorly-controlled individuals, suggesting dominance of a Th1 and weakness of Th2 immune responses. Although the present study did not include a non-diabetic group, it seems that the periodontitis development in well-controlled subjects follows the same pattern of Th1/Th2 imbalance already described for non-diabetic subjects in the literature (Shapira et al. 1992, Ukai et al. 2001, Tsai et al. 2007). In addition, we found a slight but interesting negative correlation between levels of IFN-γ and IL-17. In fact, previous

investigations have demonstrated that the Th1 cytokine, IFN-γ, may potently suppress the development of IL-17-producing Th cells from naive CD4+ precursor cells, providing a mechanism by which Th1 development could antagonize Th17 expansion (Harrington et al. 2005, Park et al. 2005).

TNF-α is a well-recognized proinflammatory cytokine in periodontitis, able to stimulate the degradation of the connective tissue matrix and bone resorption directly and indirectly (Tervahartiala et al. 2001; Kurtis et al. 2005). Although a slight significant positive correlation was found between TNF-α and HbA1c, in this study, TNF-α levels in the GCF were similar between poorly- and well-controlled subjects in all experimental periods. This finding was somewhat expected since both IFN-γ and IL-17 are able to stimulate the production of TNF-α. Findings from a recent study (Venza et al. 2010) demonstrated that TNF-α gene expression was higher in poorly-controlled (HbA1c levels ≥ 8%) than well-controlled (HbA1c levels < 8%) type 2 diabetic subjects. Such divergences in both studies may be attributed to the differences in the accuracy of the methods used to detect TNF-α.

Besides the observed clinical improvements (Table 2), in general, cytokine levels for both diabetic groups did not change significantly following the non-surgical periodontal therapy. Only a significant increase in the concentration of IFN-γ was observed at 3 and 6 months for well-controlled subjects, probably due to the reduction in GCF volume after treatment, as a result of the remission of the inflammatory process (Buduneli et al. 2009). Few studies have evaluated the effect of periodontal therapy on the local levels of inflammatory markers in diabetic subjects and conflicting results have been shown (Talbert et al. 2006, Navarro-Sanchez et al. 2007, Correa et al. 2008). Talbert et al. (2006) demonstrated no changes in the TNF-α and IL-6 levels in GCF after non-surgical treatment of periodontitis in type-2 diabetic subjects. In contrast, some studies have demonstrated that the GCF levels

of TNF-α, IL-1β, MMP-8 and MMP-9 were reduced significantly following periodontal treatment in type 2 diabetic subjects (Navarro-Sanchez et al. 2007, Correa et al. 2008). However, differences in experimental designs among studies, including the type of cytokines studied, periodontitis severity and ELISA sensitivity hampered a more meaningful comparison with the present results.

Interventional studies have evaluated the potential effects of periodontal therapies on glycemic control of diabetic subjects (Stewart et al. 2001, Kiran et al. 2005, Janket et al. 2005, Navarro-Sanchez et al. 2007, Correa et al. 2010). Therefore, one important additional finding of this study is that the levels of HbA1c did not change significantly at 3 and 6 months following non-surgical periodontal therapy for any glycemic group (Table 2). These results are in agreement with those from previous studies in which SRP resulted in periodontal clinical benefits without significant reduction in the glycemic control of diabetic subjects (Janket et al. 2005, Correa et al. 2010). On the other hand, these findings are in contrast to those from intervention studies that showed improvements in metabolic control of diabetic subjects following periodontal therapy (Stewart et al. 2001, Kiran et al. 2005, Navarro-Sanchez et al. 2007). Overall, recent meta-analyses have demonstrated positive effects of periodontal therapies in the glycemic control of diabetic subjects (Darré et al. 2008, Simpson et al. 2010, Teew et al. 2010). Darré et al. (2008) suggested that periodontal treatment could improve glycemic control after analyzing randomized and controlled interventional studies performed in type 1 and 2 diabetic subjects. Similarly, Teew et al. (2010) and Simpson et al. (2010) proposed that there may be a modest but significant improvement in glycemic control after periodontal treatment in type 2 diabetic subjects. Conflicting results among studies may be explained by differences in study designs and interventions, types of DM, initial levels of HbA1c, methods for determining

HbA1c values, severity of periodontitis, and the role of other variables on the glycemic condition such as diet, physical activity and compliance to medications and changes in hypoglycemic medications during study period. In addition, it has been recognized that a large sample size is required for the observation of any significance reduction in the HbA1c level (Janket et al. 2005, Darré et al. 2008).

In conclusion, the results of the present study indicated a trend towards a domination of pro-inflammatory Th1- or Th17-cytokines in sites of chronic periodontitis from type 2 diabetic subjects, according to their glycemic control. Since only cytokine profiles were evaluated and no characterization of T cells phenotypes in the periodontal lesions was performed, these initial findings are still not enough to define the predominant pattern of the Th immune response in each glycemic condition. Therefore, further studies are required to better characterize the Th subsets that control the periodontitis development in well- and poorly-controlled diabetic subjects. Furthermore, since Treg cells regulate the effector functions of activated Th cells, further studies are also needed to assess the role of these cells and related cytokines in the periodontal lesions of type 2 diabetic subjects and determine the impact of glycemic control in the modulation these responses.

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5. ESTUDO 3

Partial- and full-mouth scaling and root planing in type 2 diabetic subjects: a 12-month follow-up of clinical parameters and levels of cytokines and osteoclastogenesis-related factors - J Periodontal Res. 2012 Feb;47(1):45-54.

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Abstract

Objective and Background: The aim of this study was to evaluate the 12-month effects of full-mouth (FM) and partial-mouth (PM) scaling and root planing (SRP) in clinical parameters and levels of cytokines and osteoclastogenesis-related factors in type 2 diabetic subjects with chronic periodontitis.

Methods: 34 subjects received FMSRP (n=17) or PMSRP (n=17), within 24 hours or in multiple-sessions, respectively. Clinical parameters and local levels of tumor necrosis factor-α, interferon (IFN)-γ, interleukin (IL)-17, IL-23, IL-4, receptor activator of NF-κß ligand and osteoprotegerin (OPG) were assessed at baseline, 3, 6 and 12 months post-therapies.

Results: Clinical parameters improved after both therapies ($p<0.05$), without differences between groups at any time-points ($p>0.05$). Overall, there were no considerable differences in the local levels of the biomarkers studied between groups ($p>0.05$). IL-23 concentration and total IFN-γ amount increased in FMSRP and decreased in PMSRP group from baseline to 3 months and from baseline to 6 months, respectively ($p<0.05$).

Conclusion: Both PMSRP and FMSRP promoted benefits in clinical parameters and modulated the cytokines and osteoclastogenesis-related factors similarly at 12 months in type 2 diabetic subjects.

Key Words: Diabetes Mellitus; Chronic Periodontitis; Root Planing; Cytokines.

Introduction

Previous studies have shown that scaling and root planing (SRP) alone or in combination with adjunctive therapies may promote clinical periodontal improvements in diabetic subjects (1-3). Partial-mouth scaling and root planing (PMSRP), in multiple sessions with weekly intervals, is the standard protocol to treat periodontitis. However, considering the possible translocation of bacteria from one site to another in the oral cavity, it has been suggested that treated periodontal sites could be reinfected by pathogens from untreated sites when applying PMSRP (4-6). Therefore, studies have suggested that full-mouth scaling and root planing (FMSRP) within short-term could avoid this bacterial reinfection that may challenge periodontal treatment outcomes (7,8).

Periodontal breakdown is host-mediated by local production of inflammatory mediators in response to pathogens and its products (9). Several pro- [e.g. tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-17 and IL-23] and anti-[e.g. IL-4] inflammatory markers and osteoclastogenesis-related factors [e.g. receptor activator of NF-κß (RANK), RANK ligand (RANKL) and osteoprotegerin (OPG)] have been identified in the gingival crevicular fluid (GCF) as a result of cellular responses around healthy and diseased periodontal tissues (10-14). Therefore, analysis of biological markers in the GCF has been

proposed as a non-invasive means of studying the impact of systemic disorders on the pathophysiology of periodontal diseases and the host response to periodontal therapies (15).

Considering the bacterial recolonization hypothesis after employing PMSRP, FMSRP could be a better therapeutic alternative for diabetic subjects that may present an increased risk of infections. Although some studies have assessed the effect of FMSRP in diabetic subjects (2,16), to date, only one study from our research group has compared FMSRP and PMSRP in the treatment of periodontitis in type 2 diabetic subjects (3). We demonstrated that FMSRP and PMSRP were similarly effective in improving clinical parameters in diabetic subjects with chronic periodontitis at 3 and 6 months post-therapies (3). Therefore, this study aimed to compare the effects of FMSRP and PMSRP on clinical parameters and local levels of cytokines and osteoclastogenesis-related factors [soluble RANKL (sRANKL), OPG, TNF-α, IFN-γ, IL-4, IL-17 and IL-23] in type 2 diabetic subjects with chronic periodontitis over 12 months. We hypothesized that FMSRP and PMSRP for the treatment of chronic periodontitis in type 2 diabetic subjects could promote similar 12-month clinical and osteoimmunological outcomes.

Material and Methods

Sample size calculation

The sample size to assure adequate power for clinical parameter differences was calculated considering differences of at least 1 mm for clinical attachment level (CAL) and a standard deviation of 1mm between groups in initially deep pockets (≥ 7mm). Based on these calculations, it was decided that 17 subjects per group would provide an 80% power at a significance level of 5%. The number of sites for GCF sampling was based on studies that

found differences in the levels of cytokines in the GCF, when comparing clinical periodontal status and the effect of SRP (17,18).

Subject population

Thirty-four subjects (42-67 years old) with type 2 diabetes mellitus (DM) and chronic periodontitis were selected from the population referred to Guarulhos University, from December 2007 until March 2009. All eligible subjects were informed of the nature, potential risks and benefits of their participation in the study and signed their informed consent. Study protocol was previously approved by the Guarulhos University's Ethics Committee in Clinical Research.

Inclusion/ exclusion criteria

Data concerning the duration of DM and medications were retrieved from the medical records of the subjects. All subjects had type 2 DM for at least the past 5 years and generalized chronic periodontitis (19). They were under insulin supplementation, diet regimen and/or oral hypoglycemic agents. All subjects were > 30 years old, had at least 15 teeth excluding third molars and teeth indicated to exodontias, and more than 30% of the sites with probing depth (PD) and CAL ≥ 4 mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking and smoking within the past 5 years, periodontal or/and antibiotic therapies in the previous 6 months, regular use of mouthrinses containing antimicrobials in the preceding 2 months, other systemic condition that could affect the progression of periodontal disease, long-term administration of anti-

inflammatory and immunosuppressive medications, periapical pathology, orthodontic appliances and multiple systemic complications of DM.

Experimental design and treatment protocols

In this prospective, parallel, blinded, randomized and controlled clinical trial, 34 type 2 diabetic subjects with chronic periodontitis were submitted to an initial treatment phase, including supragingival plaque and calculus removals, exodontia and provisional restorations. They were instructed to use soft toothbrush, dental floss and interdental toothbrushes, as necessary, and the same brand of toothpaste during the study (Colgate Total®, Colgate-Palmolive Ind. e Com. Ltda., São Paulo, SP, Brazil).

The subjects were randomly assigned, by tossing a coin by the same assessor (PMD), to: **FMSRP** (test group, n=17): SRP was completed in 2 appointments lasting approximately 120 minutes each, under local anesthesia using periodontal curettes and ultrasonic device, in a maximum of 24 hours on two consecutive days; **PMSRP** (control group, n=17): SRP was completed in 4 appointments lasting approximately 60 minutes each, performed under local anesthesia using periodontal curettes and an ultrasonic device in a maximum of 21 days. None group received antibiotics or local antimicrobials.

Supportive therapy sessions were performed at 3, 6 and 9 months post-therapies, lasting approximately 60 minutes each. The supportive therapy included professional plaque control with abrasive sodium carbonate air-powder system, subgingival debridement, as necessary, and re-instruction of oral hygiene. The subjects were monitored by clinical parameters, fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), levels of

cytokines and osteoclastogenesis-related factors at baseline, 3, 6 and 12 months post-therapies.

Plasma and clinical monitoring

A single laboratory performed all blood analyses (Clinical Analysis Laboratory, Guarulhos University). FPG, measured by glucose oxidase method, was expressed in mg/dl. HbA1c, measured by high-performance liquid chromatography, was expressed as percentage.

Clinical examinations were performed by one examiner (VRS), calibrated according to the method described by Araujo et al. (20). The intra-examiner variability was 0.20 mm for PD and 0.23 mm for CAL. Clinical parameters registered dichotomously [e.g. bleeding on probing (BoP)] were calculated by the Kappa-Light test and the intra-examiner agreement was >0.85. The examiner and biostatician (FVR) were blinded of the treatment allocation of the subjects.

The following parameters were assessed at six sites of all teeth excluding third molars using a manual periodontal probe (North Carolina – Hu-Friedy, Chicago, IL, USA): plaque index (PI, presence/absence) (21), BoP (presence/absence), and suppuration (SUP, presence/absence), PD (mm) and CAL (mm).

GCF sampling and enzyme linked immunosorbent assay (ELISA)

Two non-contiguous sites per subject, presenting PD and CAL ≥5mm, BoP and no furcation involvement were chosen for sampling. At baseline, GCF was collected one week after clinical examination to avoid changes in its nature. GCF samples were subsequently taken from the same selected sites at 3, 6 and 12 months post-therapies. Samples were

collected and stored as previously described by Santos et al. (22). After removal of the supragingival biofilm with sterile cotton pellets, the sites were isolated with cotton rolls and gently dried with an air syringe to eliminate the possibility of contamination with saliva. GCF was collected by inserting standard paper strips (Periopaper, Oraflow Inc., Smithtown, NY, USA) approximately 2 mm into the sulcus/pocket for 30s. Strips visually contaminated with blood were discarded. The GCF sample volume was measured in a calibrated appliance (Periotron 8000, Proflow Inc., Amityville, NY, USA) and the readings were then converted to an actual volume (µl) by reference to the standard curve. The strips from the two selected sites were immediately placed into separate microcentrifuge tubes containing 250 µl phosphate-buffered saline plus protease inhibitor cocktail. The samples were stored at -20ºC for subsequent assays. GCF samples were analyzed by ELISA for TNF-α, IL-4, IFN-γ, IL-17 and IL-23 (HS Quantikine; R&D Systems Inc., Minneapolis, MN, USA), sRANKL and OPG (Biomedica Medizinprodukte GmbH & Co KG, Wien, Austria), according to the manufacturer's recommendations. The tubes were vortexed for 30s and centrifuged for 5min at 1,500xg in order to elute. The negative controls for the assays were the PBS plus protease inhibitor cocktail without GCF sample. The minimum detectable doses (sensitivity) for TNF-α, IL-4 and IL-23 range from 0.038 – 0.191 pg/ml, 0.03 - 0.22 pg/ml and 2.7 - 16.3 pg/ml, respectively. The minimum detectable doses for IFN-γ and IL-17 were < 8.0 pg/ml and < 15 pg/ml, respectively. The limit of detection for sRANKL and OPG were 1.6 pg/ml and 2.8 pg/ml, respectively. In relation to specificity, the manufacturers reported no significant cross reactivity or interference for the ELISA kits used in this study. Results were reported as total amount (pg) of each protein per site in 30s

of sampling. Calculations of the protein concentrations in each site (pg/µl) were established by dividing the total amount of the each cytokine by the GCF volume.

Statistical Analysis

Primary outcome variable was the mean CAL change post-therapies in sites with initial PD ≥7mm. Secondary outcome variables were full-mouth PI, BoP, SUP, PD, CAL and percentage of sites with PD ≥5mm, mean CAL and PD changes in the full-mouth and in sites with initial PD between 4 and 6 mm, HbA1c and FPG levels and cytokines and osteoclastogenesis related factors measurements. Data were examined for normality by Kolmogorov-Smirnov test. The data that did not achieve normality were analyzed using non-parametric methods. The percentage of sites with visible plaque, BoP, PD ≥5mm, PD ≥7mm, SUP, the mean PD, CAL, and the levels of HbA1c and FPG were computed for each subject. The changes in PD and CAL from baseline to 12 months post-therapies were examined in full-mouth level and in subsets of initial PD of 4-6mm (intermediate pockets) and ≥7mm (deep pockets). The study unit for cytokine levels was the site rather than the subject since periodontitis is a site-specific disease. Clinical parameters were averaged across subjects. Subsequently, clinical and glycemic parameters and cytokine levels and changes were averaged in each group. Clinical, glycemic, age and duration of DM differences between groups was compared by Student t-test. Cytokines levels and changes were compared between groups by Mann-Whitney *U*-test. Repeated measures ANOVA and Friedman test compared clinical/glycemic parameters and cytokine levels, respectively, within each group among time-points. A pair-wise comparison was performed by the Tukey test after ANOVA. χ^2 test was used to detect differences in the

distributions of gender, treatment regimen for DM and subjects with HbA1c >8% between groups. The level of significance was set at 5%. Adjustments were made for multiple comparisons when the levels of the seven biomarkers were evaluated. In brief, an overall p of $0.05=1-(1-k)^7$ was computed, where k was the desired individual p value. Therefore, for biomarkers levels comparisons, p values of <0.007 was considered statistically significant at $p<0.05$.

Results

There were no subject or site dropouts during the study period. Subjects from both groups reported no adverse effects such as fever and indisposition after treatment and reported no changes in the category of treatment regimen for DM during the study.

Clinical and glycemic results

No significant differences were observed between PMSRP and FMSRP groups for age, duration of DM and gender, glycemic status and treatment regimen distributions ($p>0.05$; Table 1). Table 2 presents the mean values of clinical and glycemic parameters before and after therapies. There were no differences between groups for any clinical and glycemic parameters at any time-point ($p>0.05$). Both therapies led to a significant decrease in the mean percentage of sites with PD ≥5mm, PD ≥7mm, plaque accumulation, BoP and CAL, GCF volume ($p<0.05$). PMSRP group presented significantly lower values of mean PD at 3, 6 and 12 months post-therapy when compared to baseline ($p<0.05$). Although the FMSRP group also presented reductions in means PD at 3 and 6 months, compared to baseline ($p<0.05$), at 12 months post-treatment, this parameter showed a trend towards baseline mean value. Both groups showed similar reductions in PD and CAL, considering

full-mouth and PD categories ($p>0.05$). The mean reductions in PD of intermediate pockets (PD of 4-6mm) from baseline to 12 months post-therapy were 1.5 ± 0.3 mm and 1.3 ± 0.3 mm for PMSRP and FMSRP, respectively. The mean reductions in PD of deep pockets (PD $\geq$7mm) from baseline to 12 months post-therapy were 2.5 ± 0.5 mm and 2.2 ± 0.4 mm for PMSRP and FMSRP, respectively.

Table 1 - Demographic characteristics of the study population at baseline.

Characteristics	FMSRP (n=17)	PMSRP (n=17)
Age (years)		
Mean ± SD	51.9 ± 7.8	53.1 ± 8.1
Range	42 - 66	42 - 67
Gender (n)		
Male	9	7
Female	8	10
Duration of DM		
Mean ± SD (years)	6.3 ± 0.9	6.2 ± 0.6
Glycemic status (n)		
HbA1c ≤ 8%	5	6
HbA1c >8%	12	11
HbA1c ≤ 6.5 %	1	2
HbA1c >6.5 %	16	15
Category of treatment regimen		
Diet	3	3
Diet + insulin	1	2
Diet + oral hypoglycemic agents (metformin or glybenclamin)	12	11
Diet + oral hypoglycemic agents (metformin or glybenclamin) + insulin	1	1

There were no differences between groups regarding age and duration of DM (Student t test; $p>0.05$) and regarding gender, treatment regimen and glycemic status (χ^2 test; $p>0.05$).

Table 2 - Means (± SD) o the clinical and glycemic parameters for both groups at baseline, and at 3, 6 and 12 months post-therapies

	FMSRP (n=17 subjects)				PMSRP (n=17 subjects)			
Parameters	**Baseline**	**3 months**	**6 months**	**12 months**	**Baseline**	**3 months**	**6 months**	**12 months**
PI (%)	70.9 ± 31.8a	29.2 ± 23.8b	29.2 ± 18.8b	26.5 ± 19.1b	75.4 ± 28.3a	29.3 ± 16.0b	27.0 ± 15.7b	27.2 ± 20.4b
BoP (%)	47.3 ± 26.0a	8.5 ± 7.1b	6.7 ± 10.4b	5.0 ± 5.2b	53.9 ± 36.3a	10.9 ± 10.8b	10.2 ± 12.2b	9.9 ± 11.7b
SUP (%)	1.7 ± 2.9	1.4 ± 3.2	0.3 ± 0.9	0.3 ± 1.2	3.0 ± 4.8	2.6 ± 4.2	1.0 ± 2.0	1.2 ± 1.9
PD (mm)	3.3 ± 0.9a	2.7 ± 0.9b	2.7 ± 0.5b	2.8 ± 0.5ab	3.6 ± 0.6a	2.6 ± 0.8b	2.7 ± 0.8b	2.7 ± 0.7b
CAL (mm)	3.8 ± 0.6a	3.1 ± 0.7b	3.1 ± 0.6b	3.2 ± 0.6b	4.2 ± 0.9a	3.5 ± 0.7b	3.4 ± 1.2b	3.4 ± 1.0b
GCF (µl)	0.60 ± 0.18a	0.23 ± 0.11b	0.21 ± 0.14b	0.20 ± 0.10b	0.58 ± 0.18a	0.20 ± 0.17b	0.21 ± 0.13b	0.21 ± 0.10b
Sites PD ≥ 5mm (%)	20.4 ± 8.7a	6.3 ± 3.7b	5.5 ± 4.8b	6.3 ± 4.8b	25.2 ± 11.6a	9.6 ± 7.1b	9.5 ± 8.0b	8.2 ± 6.9b
Sites PD ≥ 7mm (%)	6.4 ± 4.8 a	1.0 ± 0.8 b	1.1 ± 0.9 b	1.2 ± 1.2 b	6.9 ± 4.4 a	1.3 ± 1.0 b	1.2 ± 0.9 b	1.0 ± 1.3 b
HbA1c (%)	9.4 ± 2.5	10.1 ± 2.5	9.7 ± 2.0	10.2 ± 2.5	8.9 ± 2.2	9.3 ± 1.9	10.2 ± 2.9	10.6 ± 2.9
FPG (mg/dl)	170.8 ± 70.0	205.4 ± 87.7	199.2 ± 91.1	190.2 ± 82.8	179.4 ± 55.7	172.8 ± 56.0	198.4 ± 67.7	200.2 ± 70.0

FMSRP: full-mouth scaling and root planing; PMSRP: partial-mouth scaling and root planing; PI: plaque index; BoP: bleeding on probing; SUP: suppuration; PD: probing depth; CAL: clinical attachment level; GCF: gingival crevicular fluid; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose. For each parameter, different letters (a, b) indicate statistically significant differences among the four experimental periods within each therapeutic group (Repeated measures ANOVA and Tukey test; $p<0.05$). There were no differences between treatment groups at each time point by Student t test ($p>0.05$).

Cytokines results

There were no differences between groups regarding the levels of any biomarker at any period ($p>0.05$; Table 3). Concentrations of sRANKL were higher in the PMSRP group at 12 months and in the FMSRP group at 3 and 12 months, compared to baseline ($p<0.05$). Total amounts of OPG increased for both groups at 12 months post-therapy, when compared to the other periods ($p<0.05$). There was a significant increase in the concentrations of OPG in all time-points after PMSRP ($p<0.05$). The RANKL/OPG ratio decreased for both groups at 12 months post-therapy, when compared to the other periods ($p<0.05$). Concentrations of IFN-γ increased in PMSRP groups at 12 months post-therapy compared to baseline ($p<0.05$). IL-4 levels increased for the PMSRP group at 12 months post-therapy when compared to the other time-points ($p<0.05$). IL-17 levels decreased for both groups at 12 months after therapies when compared to baseline, 3 and 6 months ($p<0.05$). There were no changes in the levels of TNF-α and IL-23 over time ($p>0.05$; Table 3).

Table 3 - Median (range) of total amounts (pg/site) and concentrations (pg/µl) of cytokines and osteoclastogenesis-related factors for FMSRP and PMSRP groups at baseline, and at 3, 6 and 12 months post-therapies.

	FMSRP (n=34 sites)				PMSRP (n=34 sites)			
Mediators	**BASELINE**	**3 MONTHS**	**6 MONTHS**	**12 MONTHS**	**BASELINE**	**3 MONTHS**	**6 MONTHS**	**12 MONTHS**
sRANKL	44.3	39.5	38.9	42.8	42.3	36.9	40.2	40.8
(pg/site)	(18.9 - 82.6)	(20 - 66.7)	(11.5 - 86.3)	(24.3 - 91.1)	(16.3 - 137.6)	(17.8 - 219.9)	(18.9 - 120.1)	(17.3 - 65.3)
sRANKL	226.15 **a**	427.1 **b**	445.8 **ab**	1320.3 **b**	245.3 **a**	806.8 **ab**	449.9 **ab**	797.2 **b**
(pg/µl)	(44.6 - 1183.3)	(24.8 - 5718.1)	(50.2 - 5311.6)	(0.0 - 4476.5)	(25.8 - 1710.2)	(17.8 - 3221.6)	(24.2 - 5172.9)	(0.0 - 3159.5)
OPG	5.4**a**	4.8**a**	5.5**a**	7.7**b**	6.2**a**	6.6**a**	6.7**a**	10.7**b**
(pg/site)	(0.0 - 12.7)	(0.0 .- 25.1)	(0.0 - 11.6)	(0.0 - 30.6)	(0.0 - 20.9)	(2.9 - 26.0)	(1.5 - 15.4)	(1.6 - 27.7)
OPG	21.5	78.3	59.0	66.6	30.0 **a**	122.8 **b**	70.8 **b**	75.4 **b**
(pg/µl)	(0.0 - 355.6)	(0.0 - 1020.4)	(0.0 - 1065.8)	(0.0 - 339.4)	(0.0 - 291.7)	(2.5 - 928.9)	(7.5 - 632.6)	(4.4 - 903.6)
sRANKL/OPG	19.2**a**	18.9**a**	16.5**a**	4.8**b**	20.2 **a**	18.2**a**	19.54**a**	4.3**b**
	(3.0 - 128.8)	(1.8 - 63.8)	(2.0 - 81.8)	(1.6 - 81.0)	(4.1 - 150.8)	(3.7 - 37.7)	(4.1 - 19.4)	(1.5 - 22.9)
TNF-α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(pg/site)	(0.0 - 3.3)	(0.0 - 3.7)	(0.0 - 4.6)	(0.0 - 3.1)	(0.0 - 0.5)	(0.0 - 0.5)	(0.0 - 0.4)	(0.0 - 0.4)
TNF-α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(pg/µl)	(0.0 - 0.4)	(0.0 -0.4)	(0.0 - 0.3)	(0.0 - 0.9)	(0.0 - 4.8)	(0.0 - 1.7)	(0.0 -3.4)	(0.0 -1.9)
IFN-γ	3.7	6.1	7.2	7.3	5.2	5.2	4.3	8.4
(pg/site)	(0.0 - 12.5)	(0.0 - 16.9)	(0.0 - 31.3)	(0.0 - 31.3)	(0.0 - 19.3)	(0.0 - 14.2)	(0.0 - 22.7)	(0.0 - 13.4)
IFN-γ	17.8	45.8	43.8	50.8	18.2 **a**	26.6 **ab**	21.3 **ab**	43.5 **b**
(pg/µl)	(0.0 - 224.8)	(0.0 - 264.5)	(0.0 - 314.3)	(0.0 - 329.9)	(0.0 - 452.7)	(0.0 - 789.9)	(0.0 - 223.6)	(0.0 - 533.2)
IL-4	0.0	0.0	0.0	0.0	0.0 **a**	0.0 **a**	0.0 **a**	0.03 **b**
(pg/site)	(0.0 - 0.1)	(0.0 - 0.1)	(0.0 - 0.2)	(0.0 - 0.4)	(0.0 - 0.2)	(0.0 - 0.1)	(0.0 - 0.1)	(0.0 - 2.2)
IL-4	0.0	0.0	0.0	0.06	0.0 **a**	0.0 **a**	0.0 **a**	0.14 **b**
(pg/µl)	(0.0 - 0.9)	(0.0 - 3.0)	(0.0 - 3.1)	(0.0 - 4.2)	(0.0 - 3.6)	(0.0 - 3.5)	(0.0 - 1.2)	(0.0 - 12.4)
IL-17	11.0 **a**	15.5 **a**	15.5 **a**	0 **b**	13.6 **a**	13.0 **a**	14.8 **a**	0.0 **b**
(pg/site)	(0.0 - 195.5)	(0.0 - 56.8)	(0.0 - 153)	(0.0 - 42.4)	(0.0 - 88.0)	(0.0 - 183)	(0.0 - 63.0)	(0.0 - 32.4)
IL-17	46.9 **a**	80.3 **b**	144.1 **b**	0.0 **c**	61.5 **a**	82.9 **a**	70.5 **a**	0.0 **b**
(pg/µl)	(0.0 - 529.5)	(0.0 - 567.5)	(0.0 - 2266.7)	(0.0 - 392.5)	(0.0 - 880.0)	(0.0 - 1830.0)	(0.0 - 346.0)	(0.0 - 249.5)
IL-23	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
(pg/site)	(0.0 - 34.9)	(0.0 - 67.7)	(0.0 - 46.5)	(0.0 - 77.1)	(0.0 - 26.1)	(0.0 - 31.3)	(0.0 - 30.7)	(0.0 - 63.7)
IL-23	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
(pg/µl)	(0.0 - 365.9)	(0.0 - 277.0)	(0.0 - 278.0)	(0.0 - 326.2)	(0.0 - 261.0)	(0.0 - 51.2)	(0.0 - 307.0)	(0.0 - 490.3)

For each biomarker, different letters (a, b) indicate statistically significant differences among the four experimental periods within each therapeutic group (Friedman test; $p<0.05$). There were no differences between treatment groups at each time point by Mann-Whitney *U*-test ($p>0.05$).

In general, the changes in cytokine levels did not differ considerably between groups. Only the concentration of IL-23 (Fig. 1) and the total amount of IFN-γ (Fig. 2) increased in FMSRP and decreased in PMSRP from baseline to 3 months and from baseline to 6 months, respectively.

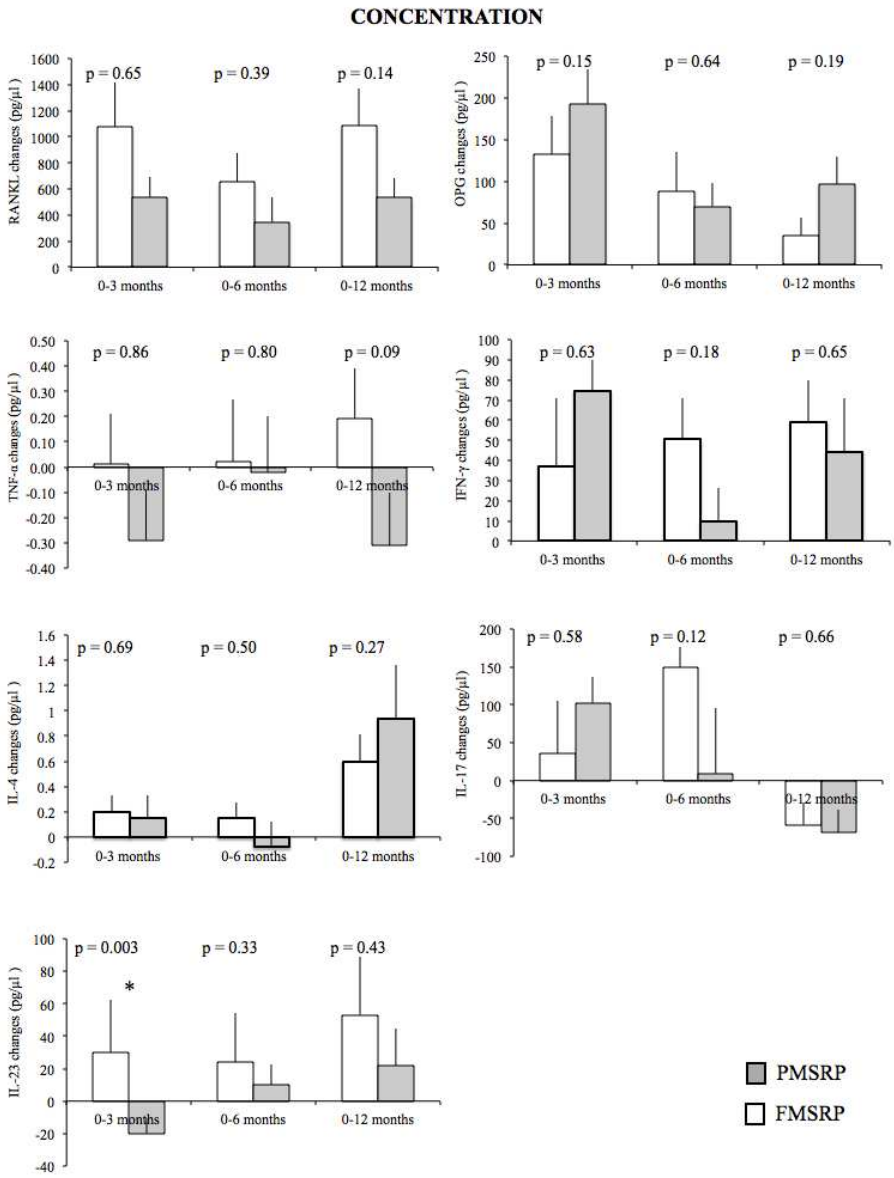

Figure 1. Bar charts of the mean changes and standard error of the mean (SEM) of the concentrations of cytokines and bone related-factors in FMSRP and PMSRP groups from baseline to 3, 6 and 12 months. * Differences between treatment groups by Mann-Whitney *U*-test ($p<0.05$). (FMSRP: full-mouth scaling and root planing; PMSRP: partial-mouth scaling and root planing)

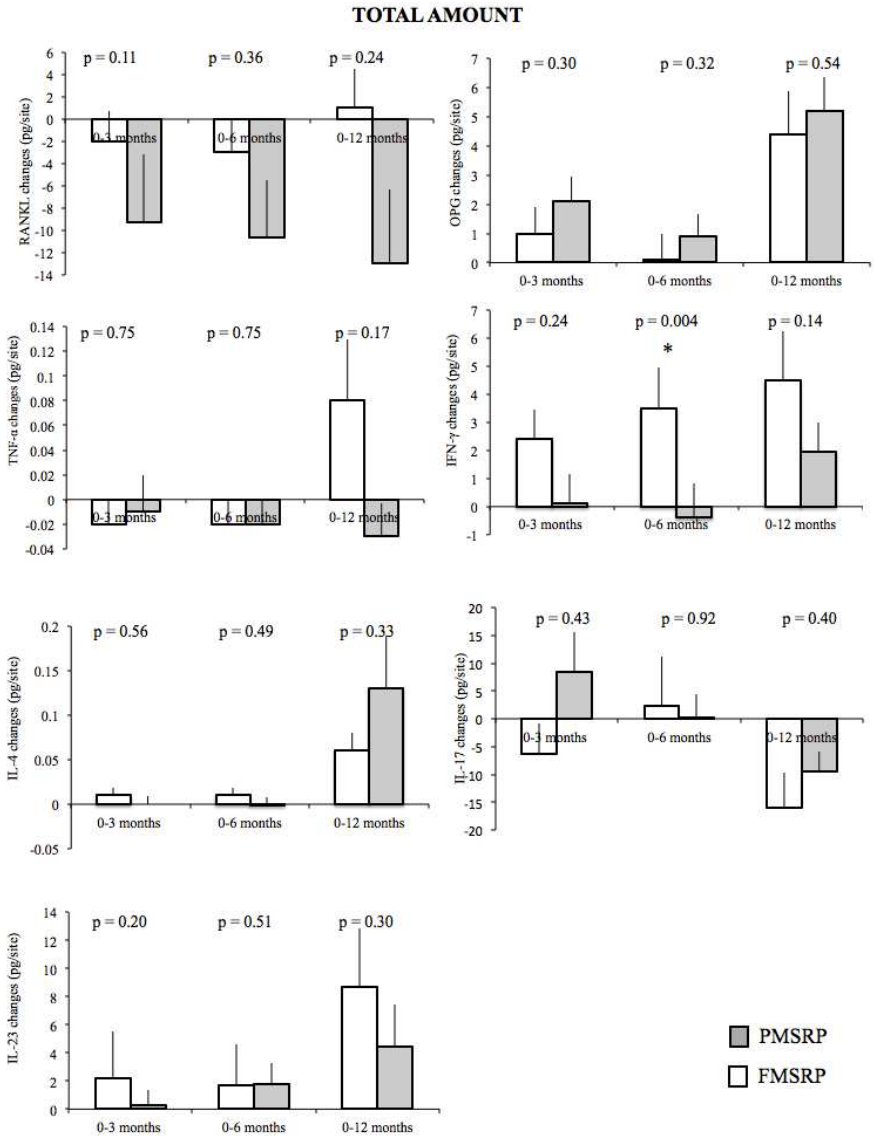

Figure 2. Bar charts of the mean changes and standard error of the mean (SEM) of the total amounts of cytokines and bone related-factors in FMSRP and PMSRP groups from baseline to 3, 6 and 12 months. * Differences between treatment groups by Mann-Whitney *U*-test ($p<0.05$). (FMSRP: full-mouth scaling and root planing; PMSRP: partial-mouth scaling and root planing)

Discussion

This is the first study to evaluate the 12-month effects of FMSRP and PMSRP on clinical outcomes and local levels of cytokines and osteoclastogenesis-related factors in type 2 diabetic subjects with chronic periodontitis. Overall, both therapies promoted improvements in clinical parameters at all periods, when compared to baseline. These findings suggest that SRP, within 24 hours or in multiple sessions, sustained comparable successful clinical outcomes during 12 months in diabetic subjects. Therefore, the hypothesis that FMSRP and PMSRP could promote comparable 12-month clinical and osteoimmunological outcomes for the treatment of chronic periodontitis in type 2 diabetic subjects was accepted. In addition, both treatment modalities were similarly able to affect the osteoimmunological profile in periodontal sites at 12 months, indicating that the control of infection by SRP modulated the levels of the biomarkers evaluated.

In this study, FMSRP and PMSRP promoted similar major clinical improvements during the initial healing phase. Subsequently, the clinical benefits achieved at 3 months were maintained until 6 and 12 months post-therapies. A previous study from our research group has already demonstrated comparable clinical improvements after FMSRP and PMSRP in type 2 diabetic subjects at 3 and 6 months (3). In the present study, no additional clinical benefits of FMSRP or PMSRP were observed at 12 months. These clinical results are in accordance with studies in non-diabetic subjects that showed short-term and mid-term improvements in periodontal parameters following FMSRP and PMSRP (8,23,24). Few studies have evaluated the effects of full-mouth therapies in non-diabetic individuals for more than 8 months (25,26), while no study has assessed the 12-month impact of these therapeutic approaches in diabetic patients. Tomasi et al. (25) revealed no difference in the

recurrence of diseased sites between the full-mouth ultrasonic debridement in one-hour session and the SRP by quadrants after 12 months. Knöfler et al. (26) demonstrated that FMSRP and PMSRP, associated with chlorhexidine, have the same clinical benefits at 12 months post-therapy. However, different treatment protocols and the absence of a diabetic status in both studies make comparisons with our results difficult.

Many investigations have evaluated the impact of periodontal therapies on the glycemic control of diabetic subjects (27-30). In this study, the levels of HbA1c and FPG did not change significantly at any time point following both periodontal therapies. Also, no differences were observed between groups for these glycemic parameters during the study. The actual effect of periodontitis on glycemic levels remains inconclusive. Janket et al. (27) proposed that SRP promotes periodontal clinical benefits without a significant reduction in the glycemic control of diabetic subjects. On the other hand, recent meta-analyses have demonstrated positive effects of periodontal therapies in the glycemic control of diabetic subjects (28-30). Contradictory results among studies about the effects of periodontal therapies on glycemic control may be attributed to differences in periodontal interventional protocols (with and without antibiotic therapies), types of DM, insufficient sample size (27), baseline HbA1c levels, severity of periodontitis and methods for determining HbA1c values (27,28). In addition, the socioeconomic profile and lifestyles including diet, physical activity, medical care and compliance to medications, which differ widely among study populations, could also interfere in the glycemic condition. Worthy of note is that the subjects included in the current investigation were predominantly from families with low socioeconomic status that often lack the financial, social and educational

supports and present inadequate or limited access to medical care, that is essential to a satisfactory glycemic control.

We also investigated whether FMSRP and PMSRP could modulate the local levels of sRANKL, OPG, TNF-α, IFN-γ, IL-4, IL-17 and IL-23 over 12 months. These mediators can stimulate and/or inhibit each other as a part of a complex network of molecules that play functions regulating the immune system and bone metabolism. Periodontal pathogens trigger several anti-microbial innate immune responses through the induction of pro-inflammatory cytokines, such as TNF-α (31). Subsequently, the innate immune response initiates the development of continuing adaptive immunity through B and T lymphocytes. CD4+T cells may differentiate into T helper (Th) 1, Th2 or Th17 phenotypes, with distinct cytokine profiles and functions (32). Th1 and Th17 cells mediate destructive inflammatory responses while the protective Th2 opposes Th1 response. The pro-inflammatory cytokines IFN-γ and IL-17 correspond to Th1 and Th17 responses, respectively, while IL-4 is anti-inflammatory Th2-cytokine. IL-23 plays a role in stabilizing the Th17 lineage and expanding Th17 responses (32). In this study, overall, both therapies affected the local levels of some biomarkers, although considerable immunological differences were not observed between groups. One could argue that the biomarkers changes could be attributed to the influence of the poor glycemic control, which is related to a systemic inflammatory burden, and not to the resolution of periodontal inflammation. However, it is important to emphasize that both groups presented comparable number of subjects with poor and good glycemic control at baseline. In addition, the mean HbA1c levels did not change significantly overtime and, both groups presented similar levels of glycemic control in all time-points.

RANKL and OPG are positive and negative regulators, respectively, of osteoclastogenesis and bone resorption (33). High levels of RANKL and low levels of OPG have been related to periodontitis (34). In this study, the concentration, but not total amount, of sRANKL increased for both groups at 12 months after therapies. However, contradictions between changes in concentration and total amount of proteins in GCF after therapies may be explained by a decrease in the GCF volume due to reduction of inflammation (11). Both therapies induced an increase in the total amount of OPG and a decrease in the RANKL/OPG ratio at 12 months, which suggest a role of OPG in controlling the periodontal bone resorption in this period. IL-17 is a pro-inflammatory cytokine, currently described in periodontitis, that stimulates the production of other pro-inflammatory mediators (35,36) and osteoclastogenesis-related factors (37,38). Levels of IL-17 were significantly higher in GCF, periodontal tissues and culture supernatants of gingival cells from periodontitis subjects (17,39). In this study, simultaneously to the increase in the levels of OPG, IL-17 was reduced significantly at 12 months after FMSRP and PMSRP. Together, these findings may indicate the stabilization of the healing process and the achievement of a healthy condition in periodontal tissues at 12 months after SRP. IL-23 is involved in the establishment of chronic inflammation and in the survival/expansion of the T helper cell subset producing IL-17 (40,41). High levels of IL-23 have been found in periodontitis lesions (39, 42). In this study, the concentration of IL-23 increased in FMSRP, while it decreased in PMSRP from baseline to 3 months (Figure 1). However, these immunological differences in the initial healing phase between therapies did not provide a notable impact on clinical outcomes overtime, and, therefore, at this stage, we were not able to determine the actual consequence of this short-term observation.

IFN-γ and IL-4 have pro- and anti-inflammatory biological functions, respectively, and, therefore, may antagonize each other (43,44). Low levels of IL-4 and high levels of IFN-γ have been related to the periodontal diseases (18,45-47). In this study, there was an increase in the concentration, but not total amount, of IFN-γ after PMSRP that could be attributed to the reduction of GCF volume after SRP, as described above (11). There was also a decrease in the total amount of IFN-γ for PMSRP and an increase for FMSRP from baseline to 6 months (Figure 2). However, we did not observe major clinical consequences of this immunological finding. Interestingly, the levels of IL-4 increased only for PMSRP at 12 months (Table 3). Since IL-4 was associated with the remission or improvement of periodontal disease (45,48), these data could suggest a favorable immunological response at 12 months following PMSRP.

In periodontitis, the excessive production of TNF-α, that plays pro-inflammatory roles (49), has been related to the loss of attachment and bone (50). In diabetic subjects, the majority of the available studies have focused on the circulating levels of TNF-α before and after periodontal therapies, with the aim of explaining the mutual relationship between DM and periodontal diseases (51,52). In this study, there were no differences regarding the levels of TNF-α following both therapies at any time point. Although a previous study (1) showed a significant reduction in the GCF concentration of TNF-α at 3 and 6 months post-PMSRP in type 2 diabetic subjects, our results are in agreement with other reports in type 2 diabetic and non-diabetic subjects in which no significant differences were found in the levels of TNF-α in GCF following SRP (10,51).

In conclusion, both PMSRP and FMSRP, for the treatment of chronic periodontitis in type 2 diabetic subjects, may improve clinical parameters and modulate local levels of cytokines and osteoclastogenesis-related factors at 12 months follow-up.

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6. ESTUDO 4

Full-mouth disinfection as a therapeutic protocol for type-2 diabetic subjects with chronic periodontitis: Twelve-month clinical outcomes. A randomized controlled clinical trial – J Clin Periodontol. 2013 Feb;40(2):155-62.

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Abstract

Aim: The aim of this randomized controlled clinical trial was to evaluate the clinical effects of chlorhexidine (CHX) application in a full-mouth disinfection (FMD) protocol in poorly controlled type-2 diabetic subjects with generalized chronic periodontitis. **Material and Methods**: Thirty-eight subjects were randomly assigned into FMD group (n=19): full-mouth scaling and root planning (FMSRP) within 24 hours + local application of CHX gel + CHX rinses for 60 days or, Control group (n=19): FMSRP within 24 hours + local application of placebo gel + placebo rinses for 60 days. Clinical parameters, glycated hemoglobin and fasting plasma glucose were assessed at baseline, 3, 6 and 12 months post-therapies. **Results:** All clinical parameters improved significantly at 3, 6 and 12 months post-therapies for both groups ($p<0.05$). There were no significant differences between groups for any clinical parameters, and glycemic condition at any time-point ($p>0.05$). **Conclusions:** The treatments did not differ with respect to clinical parameters, including the primary outcome variable (i.e. changes in clinical attachment level in deep pockets), for up to 12 months post-treatments.

Clinical Relevance

Scientific rationale for study*:** There is an interest in establishing better therapies for diabetic subjects with periodontitis, with respect to both clinical and glycemic outcomes. However, no controlled study has evaluated the effects of FMD protocol in type-2 diabetic subjects with chronic periodontitis. ***Principal findings: The FMD protocol did not provide additional clinical benefits, when compared to FMSRP alone, for the treatment of periodontitis in poorly controlled type-2 diabetic subjects up to 12 months post-therapies. ***Practical implication****:* The extensive use of CHX after FMSRP in diabetic subjects is not supported by the present study since both FMD and FMSRP can be considered similar useful therapeutic approaches.

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Introduction

Studies have long demonstrated that subjects with diabetes mellitus (DM) present a higher prevalence, extent and severity of periodontitis than non-diabetic subjects (Emrich et. al. 1991, Novak et al. 2008, Kaur et al. 2009). In addition, diabetics with poor glycemic control present more advanced periodontitis than those with adequate glycemic status (Nesse et al. 2009, Chen et al. 2010, Bandyopadhyay et al. 2010). Some investigations have also suggested a mutual relationship between DM and periodontitis in which DM may aggravate periodontal disease and, periodontitis may have a negative impact on glycemic control (Janket et al. 2005, Darré et al. 2008, Simpson et al. 2010, Teew et al. 2010). While some studies have revealed that periodontal treatment leads to an improvement in the glycemic control of diabetic subjects (Grossi et al. 1997, Kiran et al. 2005, Navarro-

Sanchez et al. 2007, Madden et al. 2008, Koromantzos et al. 2011), others have not shown meaningful positive effects of periodontal therapy on the glycemic status (Janket et al. 2005, Jones et al. 2005, Santos et al. 2009,Correa et al. 2010, Santos et al. 2012). Although scaling and root planing (SRP) alone, or in combination with adjunctive therapies (e.g. antimicrobials/antibiotics), promote clinical improvements in diabetic subjects (Grossi et al. 1997, Kiran et al. 2005, Santos et al. 2009) the actual benefits of each periodontal treatment protocol on the glycemic control of diabetic subjects are still unclear (Janket et al. 2005, Darré et al. 2008, Simpson et al. 2010, Teew et al. 2010).

Short-term full-mouth scaling and root planing (FMSRP), within 24 hours, has been proposed to overcome the possibility of bacterial translocation from untreated to already-treated periodontal pockets after conventional quadrant-wise SRP within 1- to 3-week intervals (Quirynen et al. 2000). Previous studies from our research group have demonstrated that clinical and glycemic improvements were similar after FMSRP and conventional SRP in subjects with type-2 DM and chronic periodontitis (Santos et al. 2009, Santos et al. 2012).

Chlorhexidine digluconate (CHX) was previously suggested as a potential adjunct to mechanical periodontal therapy (Quirynen et al. 1995, Quirynen et al. 2006, Faveri et al. 2006, Feres et al. 2009). Based on its broad-spectrum antiseptic action and substantivity, the use of CHX as adjunct to periodontal therapy could interfere with the subgingival microbial recolonization of recently-scaled pockets by maintaining low levels of supragingival dental biofilm and disturbing pathogen reservoirs that are not reached by SRP (i.e. tonsils, tongue, saliva, and mucosa) (Quirynen et al. 1995). In this context, FMSRP associated with sub- and supragingival application of CHX, named the “one-stage full-mouth disinfection” (FMD) protocol, was previously proposed as a suitable therapeutic

approach for the treatment of periodontitis (Quirynen et al. 1995). The principal aim of this protocol is to prevent periodontal pathogen translocation from untreated pockets, other intra-oral niches (e.g. tongue, tonsils, saliva and mucosa) and supragingival biofilm to recently-scaled sites due to the short-term SRP and the use of CHX jointly (Quirynen et al. 1995). A non-controlled study demonstrated that the FMD protocol significantly improved periodontal status and glycemic control of subjects with uncontrolled type-1 DM and periodontitis (Schara et al. 2006). However, to date, there have been no randomized controlled clinical trials (RCT) evaluating the effects of FMD in type-2 diabetic subjects with chronic periodontitis. The widespread and long-term oral application of CHX could improve the effects of SRP in diabetic subjects that present an increased risk of infections (Rajagopalan 2005). Therefore, the aim of the current study was to evaluate the clinical effects of a FMD protocol in poorly controlled type-2 diabetic subjects with generalized chronic periodontitis for up to 12 months post-therapy, when compared to FMSRP plus placebo solutions. It was hypothesized that FMD would provide greater clinical attachment gain at deep sites than FMSRP in diabetic subjects with periodontitis.

Material and Methods

Sample size calculation

The ideal sample size to assure adequate power in this study was calculated considering differences of 1.0 mm between groups for mean clinical attachment level (CAL) change in the initially deep pockets (probing depth [PD] $\geq$ 7mm) and a standard deviation of 1mm (Feres et al. 2009). Based on these data, it was determined that 16 subjects per group would be necessary to provide an 80% power with an alpha of 0.05. However, based on

anticipated attrition of approximately 15%, the number of subjects enrolled in this study was 19.

Subject population

Thirty-eight subjects (37 - 75 years old) with type-2 DM, diagnosed by a physician, and generalized chronic periodontitis were selected from the population referred to the Periodontal Clinic of Guarulhos University from July 2008 until May 2010. Detailed medical and dental records were obtained. All subjects who fulfilled the inclusion/exclusion criteria were successively invited to participate in the study. All eligible subjects were informed of the nature, potential risks and benefits of their participation in the study and signed an informed consent form. This study protocol was previously approved by Guarulhos University's Ethics Committee in Clinical Research (# 100/2007).

Inclusion and exclusion criteria

All subjects must have presented DM during the past 5 years and should be on insulin supplementation, diet regime and/or oral hypoglycemic agents (metformin or glibenclamide). Patients were diagnosed with generalized chronic periodontitis (Armitage 1999) and were ≥ 35 years old, had at least 15 teeth excluding third molars and teeth with advanced decay indicated to exodontias, and more than 30% of the sites with concomitant PD and CAL ≥4 mm at baseline.

Exclusion criteria were pregnancy, lactation, current smoking and smoking within the past 5 years, SRP in the previous 12 months, antimicrobial therapies in the previous 6 months, medical conditions requiring prophylactic antibiotic coverage, use of mouthrinses containing antimicrobials in the preceding 3 months, another systemic condition that could affect the progression of periodontitis (e.g. immunological disorders) and long-term

administration of anti-inflammatory and immunosuppressive medications. Subjects presenting allergy to CHX, periapical pathology, orthodontic appliances and major complications of DM (i.e. cardiovascular and peripheral vascular diseases [ulcers, gangrene and amputation], neuropathy and nephropathy) were also excluded.

Experimental design and treatment protocol

In this prospective, single-blinded, parallel-design, randomized, controlled clinical trial, each subject with chronic periodontitis was given a code number during the enrolment visit. The study coordinator (PMD) used a computer-generated table to randomly allocate the subjects to test (FMD: FMSRP plus CHX) or control group (FMSRP plus placebo solutions).

Initially, all subjects received one session of supragingival plaque and calculus removal, exodontia, provisional restoration and filling overhang removal, as necessary.

The subjects of the test group (n=19) were treated by a FMD protocol adapted from Quirynen et al. (2000). They received FMSRP in two appointments within 24 h, using curettes (Hu-Friedy, Chicago, IL, USA) and an ultrasonic device (Cavitron Select SPC, Dentsply professional, York, PA, USA). Immediately after each instrumentation appointment, the mouth was washed for 1 min with a 0.12% CHX solution (Proderma Farmácia de Manipulação Ltda, Piracicaba, SP, Brazil) and all treated pockets were irrigated with a 1% CHX gel (ProdermaFarmácia de ManipulaçãoLtda) three times (repeated within 10 min). The dorsum of the tongue was brushed with the same gel for 1 min. The subjects were instructed to rinse 15ml of 0.12% CHX solution for 50s and gargle for 10s, twice daily, 12 hours apart, for 60 days.

The control group (n=19) was submitted to the same protocol as described for the test group. However, placebo gel and rinse were applied (Proderma Farmácia de Manipulação

Ltda). The placebo solutions were mainly composed of aspartame, zinc acetate dehydrate, alcohol, colorant, flavoring, acesulfame and purified water. The test solutions were composed of the same components of placebo solutions plus chlorhexidine digluconate.

Identical plastic bottles containing the placebo or CHX gels and rinses were sent to the study coordinator, who marked the code number of each subject on each bottle, according to the therapy assigned. The coded bottles were given to the operator (JAL), who at no time during the study identified the contents of the bottle or subject assignment to therapies. In addition, the biostatistician (MF) and volunteers were also blinded to treatment assignment.

All subjects were instructed to perform a brushing technique using a soft toothbrush, dental floss and interdental toothbrushes, as necessary, and received the same brand of toothpaste during the study (Colgate Total®, Colgate Palmolive Co., São Paulo, SP, Brazil). Systemic antimicrobials were not used. Supportive therapy sessions were performed at 3, 6 and 9 months post-therapies including professional plaque control with abrasive sodium carbonate air-powder system, subgingival debridement, as necessary, and re-instruction of oral hygiene.

Clinical and glycemic monitoring

The Clinical Analysis Laboratory of Guarulhos University performed all blood analyses. All clinical parameters, fasting plasma glucose (FPG), glycated hemoglobin (HbA1c) were monitored at baseline, 3, 6 and 12 months post-therapies. FPG (mg/dl) was measured by glucose oxidase method and HbA1c (%) was obtained by high-performance liquid chromatography.

Clinical examinations were performed by one examiner (VRS), calibrated as previously described (Santos et al. 2012). The intra-examiner variability was 0.20 mm for PD and 0.23 mm for CAL. Clinical parameters were registered dichotomously [e.g. bleeding on probing

(BoP)] and calculated by the Kappa-Light test; the intra-examiner agreement was >0.85. Plaque index (PI) (Ainamo & Bay, 1975), BoP (presence/absence), suppuration (SUP, presence/absence), PD (mm) and CAL (mm) were assessed at six sites per tooth excluding third molars using the manual periodontal probe (North Carolina - Hu-Friedy, Chicago, IL, USA). The examiner was unaware of the treatment allocation of the subjects. However, since continuing CHX rinsing could stain the tooth/tongue surfaces, the examiner could deduce the subjects that were receiving CHX or placebo solutions. For this reason, this study was not considered double-blinded.

Compliance and monitoring of adverse events

Assistants (TSM and TEDG) monitored the compliance to rinses by calling the patients every week during the 60 days. In addition, the subjects were asked to bring the empty bottles back every 15 days, and get a new bottle containing placebo or CHX solutions. Therefore, each subject received a total of 4 bottles of placebo or CHX rinses (500 ml each) during the experiment. All subjects also answered a questionnaire (administered by a study assistant) about any self-perceived side effects of the mouthwashes on the 60th day.

Statistical Analysis

The primary outcome variable was the difference between groups at 12 months for the mean CAL change in sites with initial PD ≥ 7mm. Secondary outcomes included the additional clinical measurements and the glycemic parameters. The percentage of sites with plaque accumulation, BoP, SUP, PD ≥ 5mm, PD ≥ 7mm, the mean PD, CAL and the levels ofHbA1c and FPG were computed for each subject. The changes in PD and CAL from baseline to 3, 6 and 12 months were evaluated according to the initial moderate (PD 4-6 mm) and deep (PD≥ 7 mm) pockets for both groups. Clinical parameters were averaged across subjects and in both groups. HbA1c and FPG data were averaged for each group.

Data were examined for normality by the Kolmogorov-Smirnov test and were analyzed using parametric methods. The significance of differences between the test and control groups for age, duration of DM, clinical and glycemic parameters at each time-point were compared by Student’s t-test. Repeated measures ANOVA and Tukey test were used to detect differences within each group over time. The Chi-square test was used to detect differences in the frequencies of gender and DM regimen between groups. Fisher's exact test compared the frequency of subjects according to changes in the HbA1c from baseline to the follow-up examinations. The level of significance was set at 5%.

Results

Subject retention

Figure 1 presents the flow chart of the study design. Of the 38 subjects selected, one from the control group did not return for the 3-month follow-up visit and was not included into the statistical analysis. A total of 37 subjects were monitored at 3 and 6 months; 19 of these were in the test group and 18 were in the control group. Two subjects per group did not return for the twelve-month visit. Therefore, intention-to-treat analyses were performed in these four subjects (their 6-month data were carried forward). The subjects from both groups reported no adverse effects such as fever and indisposition after treatment. All subjects informed full adherence to the use of rinses. Seventeen subjects from the FMD group and 12 from control group reported one or two adverse events to the rinses including changes in taste perception, dry mouth and staining. The subjects reported no changes in the category of DM treatment regimen during the study.

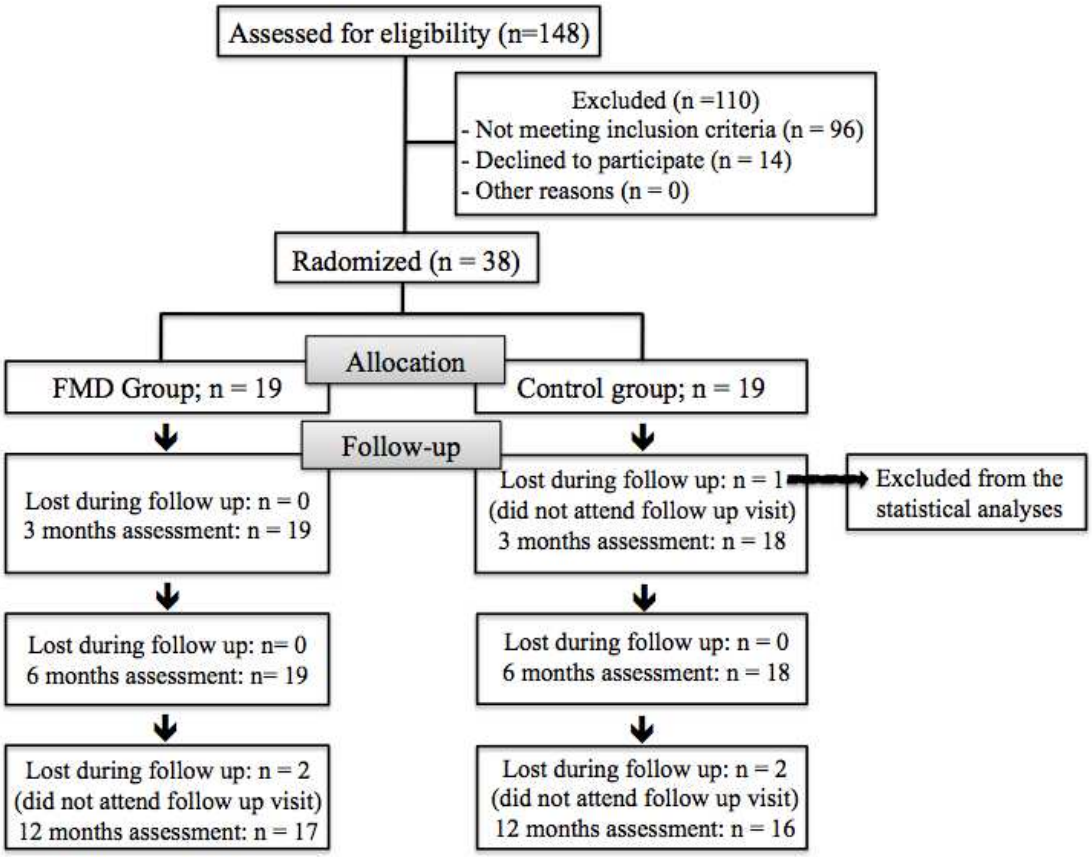

Figure 1 - Flow chart of the study design.

Clinical and glycemic results

There were no significant differences between groups for age, gender, duration of DM, glycemic status and category of DM regimen (Table 1; $p>0.05$).

Table 1 - Demographic characteristics of the study population at baseline.

Characteristics	FMD group (n=19)	Control group (n=19)
Age (years)		
Mean ± SD	50.3 ± 9.5	53.9 ± 10.8
Range	37 - 70	35 - 75
Gender (M/F)	4/15	6/13
Duration of DM		
Mean ± SD (years)	6.3 ± 0.8	6.8 ± 1.1
Glycemic status (n)		
HbA1c ≤ 8%	4	6
HbA1c >8%	15	13
DM regimen category		
Diet	1	0
Diet + insulin	1	4
Diet + oral hypoglycemic agents	14	14
Diet + oral hypoglycemic agents + insulin	3	1

There were no differences between groups using Student's t-test or the Chi-square test (p>0.05)

Table 2 presents the mean full-mouth values and the glycemic parameters at baseline, 3, 6 and 12 months post-therapies. There were no significant differences between groups for any clinical and glycemic parameters at any time point (P>0.05). Both therapies led to significant reductions in the mean percentage of sites with plaque accumulation and BoP and, mean PD and CAL at 3, 6 and 12 months, compared to baseline (p<0.05). There were no significant changes in the HbA1c and FPG levels over time for any group (p>0.05).

Table 2 - Means (± SD) of the clinical and glycemic parameters for both groups at baseline, 3, 6 and 12 months post-therapies.

		FMD group (n=19)	**Control group** (n=18)
Number of teeth	Baseline	20.0 ± 3.7	19.6 ± 3.4
Plaque accumulation (%)	Baseline	65.9 ± 19.1[a]	58.8 ± 23.6 [a]
	3 months	35.0 ± 13.1[b]	31.8 ± 13.9[b]
	6 months	36.9 ± 15.0[b]	27.5 ± 12.4[b]
	12 months	34.5 ± 12.2[b]	30.0 ± 15.9[b]
BoP (%)	Baseline	33.9 ± 18.2[a]	31.3 ± 22.4[a]
	3 months	17.4 ± 14.9[b]	15.7 ± 11.3[b]
	6 months	14.5 ± 11.9[b]	10.7 ± 9.9[b]
	12 months	16.7 ± 14.6[b]	10.5 ± 7.5[b]
SUP (%)	Baseline	1.7 ± 3.8	0.5 ± 0.9
	3 months	0.5 ± 1.3	0.1 ± 0.2
	6 months	0.9 ± 2.4	0.5 ± 1.4
	12 months	0.2 ± 0.5	0.6 ± 1.5
PD (mm)	Baseline	3.4 ± 0.5[a]	3.7 ± 0.8[a]
	3 months	2.9 ± 0.4[b]	3.0 ± 0.5[b]
	6 months	2.9 ± 0.4[b]	2.9 ± 0.5[b]
	12 months	3.0 ± 0.4 [b]	2.9 ± 0.6[b]
CAL (mm)	Baseline	4.1 ± 0.85[a]	4.4 ± 0.99 [a]
	3 months	3.7 ± 0.92[b]	3.9 ± 0.87 [b]
	6 months	3.6 ± 0.77[b]	3.8 ± 0.92 [b]
	12 months	3.9 ± 0.90[b]	3.8 ± 0.81[b]
HbA1c (%)	Baseline	10.0 ± 2.41	10.4 ± 2.9
	3 months	9.3 ± 2.75	9.7 ± 2.9
	6 months	9.9 ± 2.40	9.6 ± 3.2
	12 months	9.7 ± 2.54	8.9 ± 2.5
FPG (mg/dl)	Baseline	175.4 ± 52.3	186.4 ± 80.7
	3 months	170.0 ± 72.9	185.0 ± 73.4
	6 months	169.9 ± 61.4	171.0 ± 79.4
	12 months	168.2 ± 82.6	165.8 ± 77.5

FMD: full-mouth disinfection; BoP: bleeding on probing; SUP: suppuration; PD: probing depth; CAL: clinical attachment level; HbA1c: glycated hemoglobin; FPG: fasting plasma glucose. Different letters indicate significant differences over time by repeated measures ANOVA and Tukey test ($p<0.05$). There were no differences between groups at any time point by Student's t-test ($p>0.05$).

The FMD and control groups presented similar reductions in PD and CAL from baseline to 3, 6 and 12 months post-therapies, considering both moderate and deep PD categories (Table 3; $P>0.05$).

Table 3 - Mean reductions (±SD) of probing depth (PD) and clinical attachment level (CAL) at sites with initially moderate (PD 4-6 mm) and deep PD (PD ≥ 7mm) from baseline to 3, 6 and 12 months.

Parameter	PD category	Time point	Treatment groups		*P* value
			FMD group	**Control group**	
PD	Moderate	0-3 months	1.1 ± 0.5	1.2 ± 0.3	0.27
		0-6 months	1.1 ± 0.5	1.2 ± 0.5	0.71
		0-12 months	1.0 ± 0.6	1.2 ± 0.6	0.38
	Deep	0-3 months	2.4 ± 1.0	2.6 ± 1.0	0.52
		0-6 months	2.4 ± 0.8	3.1 ± 1.1	0.16
		0-12 months	2.4 ± 1.1	3.1 ± 1.3	0.13
CAL	Moderate	0-3 months	0.8 ± 0.6	0.9 ± 0.4	0.31
		0-6 months	0.9 ± 0.4	0.9 ± 0.5	0.57
		0-12 months	0.8 ± 0.5	0.9 ± 0.7	0.69
	Deep	0-3 months	1.8 ± 1.0	2.0 ± 0.9	0.54
		0-6 months	1.7 ± 0.9	2.4 ± 0.8	0.10
		0-12 months	1.7 ± 1.2	2.4 ± 1.0	0.28

There were no differences between groups by Student's t-test ($p>0.05$).

The mean percentage and number of sites with PD<5 mm, PD ≥5 mm and PD ≥7 mm are presented in Table 4. There were significant reductions in the percentage and number of sites with PD ≥5 mm at all follow-up examinations compared to baseline ($p<0.05$), with no differences between groups ($p>0.05$) at all time-points. Furthermore, the mean percentage and number of sites with initial PD≥5 mm that was reduced by at least 2mm in CAL and PD, concomitantly, were similar in both groups at all time-points.

The mean reductions in HbA1c and the distribution of subjects according to changes in HbA1c are presented in Table 5. An increase or decrease of ≥ 0.5% in HbA1c from baseline to the follow-up examinations was considered a change, otherwise the level of glycemic control was considered stable. There were no differences between groups in mean reduction and frequencies of subjects that presented decreases, increases or no changes in HbA1c levels from baseline to 3, 6 and 12 months ($p>0.05$).

Table 4 - Mean percentage and mean number (± SD) of sites with PD <5mm, PD ≥5 mm with and without BoP and PD ≥7 mm.

PD category	Time point	Treatment groups	
		FMD group mean % (mean number)	**Control group** mean % (mean number)
PD< 5mm	Baseline	80 ± 10.8 (97.8 ± 27.7) [a]	73.6 ± 18.2 (87.1 ± 29.7) [a]
	3 months	89.8 ± 7.0 (108.6 ± 25.5) [b]	88.4 ± 10.6 (102.9 ± 22.3) [b]
	6 months	90.7 ± 6.3 (109.9 ± 25.2) [b]	88.8 ± 9.9 (103.5 ± 23.6) [b]
	12 months	80.9 ± 6.8 (108.2 ± 25.6) [b]	88.7 ± 10.7 (103.5 ± 24.4) [b]
PD ≥ 5mm	Baseline	19.8 ± 10.8 (22.5 ± 10.9) [a]	26.4 ± 18.2 (30.2 ± 22.7) [a]
	3 months	10.2 ± 7.0 (11.4 ± 6.9) [b]	11.6 ± 10.6 (13.5 ± 13.1) [b]
	6 months	9.3 ± 6.3 (10.4 ± 6.4) [b]	11.2 ± 9.9 (12.6 ± 11.1) [b]
	12 months	10.8 ± 6.8 (12.7 ± 11.9) [b]	11.3 ± 10.7 (12.7 ± 11.9) [b]
PD ≥ 5mm (with BoP)	Baseline	12.7 ± 8.6 (14.6 ± 9.4) [a]	16.5 ± 16.1 (19.2 ± 22.1) [a]
	3 months	4.8 ± 3.3 (5.4 ± 3.3) [b]	4.4 ± 5.7 (5.5 ± 8.1) [b]
	6 months	3.8 ± 4.5 (4.2 ± 4.6) [b]	4.1 ± 4.5 (4.8 ± 5.4) [b]
	12 months	4.8 ± 4.3 (5.3 ± 4.2) [b]	4.1 ± 4.1 (4.8 ± 5.4) [b]
PD ≥ 7mm	Baseline	4.4 ± 5.3 (4.8 ± 5.6) [a]	8.5 ± 10.8 (9.5 ± 12.0) [a]
	3 months	1.5 ± 2.3 (1.7 ± 2.5) [b]	2.6 ± 3.0 (3.0 ± 3.3) [b]
	6 months	1.6 ± 2.0 (1.9 ± 2.4) [b]	3.0 ± 3.8 (3.3 ± 4.3) [b]
	12 months	1.8 ± 2.2 (2.0 ± 2.5) [b]	2.9 ± 4.0 (3.3 ± 4.5) [b]
Sites with initial PD ≥ 5mm that reduced at least 2mm in CAL and PD concomitantly		_____	_____
	0-3 months	12.4 ± 7.5 (14.4 ± 8.2)	15.5 ± 11.7 (17.6 ± 14.7)
	0-6 months	12.1 ± 6.2 (14.0 ± 6.3)	17.6 ± 12.0 (19.9 ± 15.3)
	0-12 months	11.4 ± 5.9 (13.4 ± 6.5)	17.0 ± 11.8 (19.1 ± 14.7)

FMD: full-mouth disinfection; PD: probing depth; BoP: bleeding on probing. CAL: clinical attachment level. Different letters indicate significant differences over time by repeated measures ANOVA and Tukey test ($P<0.05$). There were no differences between groups at any time point by Student's t-test ($P>0.05$).

Table 5 - Mean reductions and frequencies of subjects according to changes in HbA1c from baseline to 3, 6 and 12 months for FMD and control groups.

	FMD group (n=19)			**Control group** (n=18)		
	0-3 months	0-6 months	0-12 months	0-3 months	0-6 months	0-12 months
Mean reduction (%)	0.78 ± 3.5	0.19 ± 2.9	0.35 ± 3.4	0.68 ± 2.5	0.74 ± 4.1	1.4 ± 3.0
Decrease (n)	9	6	12	11	10	13
Increase (n)	6	12	6	5	8	5
No changes (n)	4	1	1	2	0	0

An increase or decrease of ≥ 0.5% in HbA1c from baseline to the other follow-up examinations was considered a change; otherwise the level of glycemic control was considered stable. There were no differences between groups for mean reduction in HbA1c at any time point by Student's t-test (P>0.05). There were no differences between groups in the distribution of subjects according to changes in HbA1c by Fisher's exact test (P>0.05).

Discussion

There is an increasing interest in establishing more effective therapies to improve periodontal and glycemic conditions in diabetic subjects with periodontitis. It has been proposed that FMD, including short-term SRP and extensive oral application of CHX, could be especially useful for diabetic subjects that present a higher risk for infection and possible cross-contamination (Rajagopalan 2005). As such, this is the first controlled trial evaluating clinical and glycemic effects of a FMD protocol for generalized chronic periodontitis in a population of type-2 diabetic subjects presenting a poor glycemic control predominantly. Although both therapies provided clinical improvements, the adjunctive application of CHX had no additional benefits over FMSRP within 24h without use of

antiseptic for up to 12 months in diabetic subjects. Therefore, the hypothesis that the FMD would provide better clinical improvements in diabetic subjects was rejected.

In this study, there were significant improvements in periodontal parameters for both therapies, including reductions in plaque accumulation, BoP, SUP, PD, CAL and number of sites with PD ≥5 mm and PD ≥7 mm at 3, 6 and 12 months post-therapies, when compared to baseline (Table 2, 3 and 4). Furthermore, although the use of CHX in the FMD protocol is proposed to prevent re-infection of pathogens from non-dental niches and supragingival plaque to already-treated pockets (Quirynen et al. 1995), SRP with and without CHX did not differ regarding clinical benefits at any time-point. These findings are in agreement with previous studies in non-diabetic subjects (Quirynen et al. 2000, Swierkot et al. 2009), which showed that FMD did not yield additional clinical benefits over short-term FMSRP at 4 and 8 months post-therapies. Previous investigations (Quirynen et al. 1995, Quirynen et al. 2000) have demonstrated some clinical and microbiological advantages of the use of 0.2% CHX for 14 days and 60 days during the initial healing phase (1st and 2nd months) after FMD. However, these benefits were not maintained in long-term evaluations (4th and 8th months post-treatment) (Quirynen et al. 2000), as noticed in the present study. Systematic reviews proposed that FMD presented modest advantages over conventional SRP in the treatment of chronic periodontitis (Eberhard et al. 2008; Lang et al. 2008). However, as observed in the present study, they demonstrated that FMD approach yielded no additional advantages over FMSRP. Instead, unexpectedly, these reviews noted that the comparison between FMD and FMSRP demonstrated some slight clinical benefits in favor of FMSRP. In the present study, there was also a trend, although not statistically significant, towards greater reductions in mean PD and CAL in moderate and deep pockets from baseline to 3, 6 and 12 months for the control compared to the FMD

group (Table 3). However, it is important to observe that, although both groups were statistically similar at baseline, the control group presented a worse initial periodontal condition in relation to mean full-mouth PD, CAL and number of pockets with PD ≥5mm and PD ≥ 7mm. Since it has been demonstrated that greater clinical improvements occur in deeper sites, it is possible that the subtle advantage of FMSRP over FMD may be attributed to these baseline differences. Unexpectedly, the control group also presented a trend to have 4 times greater reduction in the mean HbA1c levels from baseline to 12 months than FMD group (Table 5). However, at this stage, it is not possible to assure the actual effects of the abovementioned clinical improvements on this glycemic finding of control group. In addition, it is important to highlight the possible advantageous effects of the supportive therapy applied during the study on the clinical results of both groups. Subjects under FMD and control therapies were under supportive therapies at 3, 6 and 9 months, in which they received professional plaque control, subgingival debridement, as necessary, and re-instruction of oral hygiene. Previous studies have demonstrated significant clinical improvements in diabetic subjects after FMD (Schara et al. 2006) and SRP associated with the use of the CHX rinse (Madden et al. 2008). However, these studies did not include a control group and presented marked differences in treatment protocol, study design and the type of DM, when compared to our study.

Previous studies have demonstrated positive effects of the periodontal therapy on the glycemic parameters of diabetic subjects (Grossi et al. 1997, Kiran et al. 2005, Navarro-Sanchez et al. 2007, Madden et al. 2008, Koromantzos et al. 2011). In this study, 12 and 13 subjects from the FMD and control groups, respectively, presented a decrease of ≥ 0.5% in HbA1c at 12 months post-therapies (Table 5). In addition, there was a trend towards reduction in the levels of HbA1c after both therapies in all experimental periods (Table 2).

However, the FMSRP with or without the application of CHX yielded no statistically significant benefits for the glycemic control of the subjects. Therefore, some limitations of the present study regarding the glycemic effects of the periodontal therapies should be mentioned. First, our sample size calculation was based on a periodontal primary outcome variable (mean CAL changes in deep sites) while the changes in glycemic parameters were considered as secondary outcome variables. Consequently, as previously suggested (Janket et al. 2005, Darré et al. 2008), it is possible that a larger sample size would be necessary to observe any significant change, if it exists, in the HbA1c levels after the therapies studied. Second, as previously discussed (Santos et al. 2012), the diabetic subjects from our study center generally have a limited access to adequate diet, medication and medical care, which may directly affect their glycemic control rather than the periodontal infection and the possible benefits of the periodontitis treatment. The negative influence of these medical and behavioral factors can be confirmed by the poor control of DM observed for the majority of the participants involved in the present study (Table 1). Studies have reported greater improvements in the glycemic condition of diabetic subjects when systemic antibiotics are used as adjunct to SRP, suggesting that the impact of periodontal treatment on glycemia may vary according the type of therapy (Grossi et al. 1997, Janket et al. 2005, O'Connell et al. 2008). However, to date, there are insufficient data regarding the effects of the use of CHX as an adjunct to SRP (Grossi et al. 1997, Madden et al. 2008) and the FMD (Schara et al. 2006) protocol on glycemia. Schara et al. (2006), for example, showed that FMD significantly improved the glycemia of uncontrolled type-1 diabetic subjects at 3 months post-therapy. However, in addition to the difference in the DM type, a control group was not included in that study.

Conclusion

FMD and FMSRP plus placebo solutions for the treatment of type-2 diabetic subjects with chronic periodontitis did not differ with respect to clinical parameters, including the primary outcome variable (i.e. changes in clinical attachment level in deep pockets), for up to 12 months post-treatments

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7. CONSIDERAÇÕES FINAIS

ESTUDO 1 – Estudos sugerem que a proporção RANKL/OPG é mais elevada em sítios com doenças periodontais quando comparado à sítios saudáveis, e que o desequilíbrio nesta proporção apresenta um papel importante na perda óssea decorrente da periodontite. Este estudo avaliou os níveis de RANKL, OPG e RANKL/OPG no fluido gengival de indivíduos portadores de periodontite crônica com DM tipo 2 com controle glicêmico inadequado e satisfatório, antes e após terapia periodontal básica. Os resultados demonstraram que a proporção RANKL/OPG em sítios periodontais tratados e não tratados pode ser negativamente influenciada pelo controle glicêmico precário em diabéticos tipo 2. Sendo assim, a reabsorção óssea periodontal pode ser afetada pelo estado glicêmico de diabéticos tipo 2.

ESTUDO 2 - Existe uma escassez de estudos científicos que avaliaram o perfil imuno-inflamatório de indivíduos diabéticos tipo 2 com periodontite crônica, principalmente, levando em consideração o controle glicêmico. O delineamento do aspecto imuno-inflamatório periodontal em diabéticos é importante para um melhor entendimento da destruição clínica exacerbada nestes indivíduos e para a proposição de terapias específicas para esse grupo de risco. Os resultados deste estudo indicaram um predomínio de citocinas próinflamatórias relacionadas às respostas Th1 e Th17 em sítios com periodontite crônica de acordo com o controle glicêmico de indivíduos diabéticos tipo 2.

ESTUDO 3 - Considerando a hipótese de re-colonização bacteriana após a realização de RAR por quadrante, foi sugerido que a RAR de boca-toda em curto tempo poderia ser uma

alternativa terapêutica melhor para indivíduos diabéticos que apresentam um risco elevado para infecções. Esse estudo avaliou os efeitos da RAR em curto prazo e por quadrantes nos parâmetros clínicos e nos níveis locais de citocinas (TNF-α, INF-γ, IL-17, IL-23 e IL-4) e fatores relacionados a osteoclastogênese (RANKL e OPG) em indivíduos diabéticos tipo 2 com periodontite crônica. Ambas as terapias promoveram benefícios semelhantes nos parâmetros clínicos e na modulação de citocinas e fatores relacionados à osteoclastogênese, em 12 meses pós-terapias.

ESTUDO 4 – Com o objetivo de ampliar os efeitos da RAR de boca-toda em curto prazo de tempo e atingir patógenos presentes em sítios não-dentais, estudos têm sugerido a utilização do protocolo de desinfecção de boca-toda em estágio único (RAR em curto prazo de tempo associada à extensa aplicação oral de clorexidina). Este estudo avaliou os efeitos do uso da clorexidina no protocolo de desinfecção de boca-toda, no tratamento de diabéticos tipo 2 com periodontite crônica. Os resultados demonstraram que, em 12 meses, o uso da clorexidina no protocolo de desinfecção de boca-toda, não apresentou benefícios adicionais sobre a RAR de boca-toda somente.

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OUTRAS PRODUÇÕES DO DOUTORADO

Santos VR, Lima JA, Miranda TS, Feres M, Zimmermann GS, Nogueira-Filho Gda R, Duarte PM. Relationship between glycemic subsets and generalized chronic periodontitis in type 2 diabetic Brazilian subjects. Arch Oral Biol. 2012 Mar;57(3):293-9. Epub 2011 Sep 3. PubMed PMID: 21890108.

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ANEXO A

Guarulhos, 08 de agosto de 2007.

Exma. Sra.
Profa. Poliana Mendes Duarte

PARECER Nº 100/2007

Referência: **Aprovação de Projeto**
SISNEP/277 - "Raspagem a alisamento radicular em até 24 horas ou por quadrantes em 4 semanas em pacientes com periodontite crônica portadores de diabetes mellitus: avaliação clínica, microbiológica e do controle metabólico"

O Comitê de Ética em Pesquisa da Universidade Guarulhos analisou o Projeto de Pesquisa de sua autoria "Raspagem a alisamento radicular em até 24 horas ou por quadrantes em 4 semanas em pacientes com periodontite crônica portadores de diabetes mellitus: avaliação clínica, microbiológica e do controle metabólico" - SISNEP/277, na reunião de 08.08.2007, e no uso das competências definidas na Res. CNS 196/96, considerou o Projeto acima **aprovado**.

As orientações abaixo devem ser consideradas pelo Pesquisador Responsável durante a realização da pesquisa, visando que a mesma se desenvolva respeitando os padrões éticos:

- O sujeito da pesquisa tem a liberdade de recusar-se a participar ou de retirar seu consentimento em qualquer fase da pesquisa, sem penalização alguma e sem prejuízo ao seu cuidado e deve receber uma cópia do Termo de Consentimento Livre e Esclarecido, na Integra, por ele assinado.
- O pesquisador deve desenvolver a pesquisa conforme delineada no protocolo aprovado e descontinuar o estudo somente após análise das razões da descontinuidade pelo CEP que o aprovou, aguardando seu parecer, exceto quando perceber risco ou dano não previsto ao sujeito participante ou quando constatar a superioridade de regime oferecido a um dos grupos da pesquisa que requeiram ação imediata.
- Eventuais modificações ou emendas e eventos adversos ao protocolo, devem ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada e suas justificativas.
- Esclarecemos a necessidade da apresentação de relatório de andamento I até **15.08.08,** relatório de andamento II até **15.08.09,** e final até **15.09.10.**

Luciene Cristina de Figueiredo
Coordenadora do Comitê de Ética em Pesquisa