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FACULDADE DE ODONTOLOGIA DE PIRACICABA

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Orientador: Profa. Dra. Cristiane Duque
Co-orientador: Profa. Dra. Renata de Oliveira Mattos Graner

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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Biologia Buco-Dental da Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para obtenção do título de Mestra em Biologia Buco-Dental, área de Microbiologia e Imunologia.

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Este exemplar corresponde à versão final da Dissertação defendida pela aluna Mariana Ferreira Dib João e orientada pela Prof. Dr. Cristiane Duque

Assinatura do Orientador
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A Comissão Julgadora dos trabalhos de Defesa de Dissertação de Mestrado, em sessão pública realizada em 18 de Outubro de 2013, considerou a candidata MARIANA FERREIRA DIB JOÃO aprovada.

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Profa. Dra. ANA CLAUDIA OKAMOTO

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Prof. Dr. JOSE FRANCISCO HOFLING

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RESUMO

A doença periodontal compreende um grupo de infecções inflamatórias que afeta os tecidos periodontais, podendo ser desencadeada por múltiplos fatores locais e sistêmicos. Indivíduos de todas as faixas etárias são susceptíveis ao desenvolvimento dessa doença e a gengivite, condição reversível limitada aos tecidos gengivais, é comumente vista em crianças e no início do período da adolescência. A presença de uma microbiota patogênica específica é um fator essencial para o desenvolvimento da doença periodontal (DP). Doenças sistêmicas tais como diabetes melito, podem contribuir para o desenvolvimento da doença, principalmente devido à resposta exacerbada do sistema imunológico e alterações em parâmetros fisiológicos que contribuem para a agressão tecidual. O objetivo deste estudo foi comparar os aspectos clínico, microbiológico e imunológico de crianças portadoras de diabetes melito tipo I (DM) com crianças não diabéticas (NDM). Vinte e quatro pacientes diabéticos e vinte e sete normoglicêmicos foram avaliados. As condições bucais foram avaliadas através dos índices de placa, gengival e profundidade de sondagem e o perfil de saúde geral dos pacientes foi avaliado através dos níveis de glicemia, HbA1c (hemoglobina glicosilada), triglicerídeos (TRG), colesterol total (CT), HDL, LDL, VLDL e lipídeos totais (LT), a partir de amostras sanguíneas. O método do PCR foi utilizado para identificação das seguintes bactérias: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td) (complexo vermelho), *Prevotella intermedia* (Pi) e *Prevotella nigrescens* (Pn), *.Fusobacterium nucleatum* (Fn), *Campylobacter rectus* (Cr) (complexo laranja), *Capnocytophaga sputigena* (Cs), *Capnocytophaga ochracea* (Co), *Eikenella corrodens* (Ec) (complexo verde) em amostras do sulco gengival de dentes decíduos e permanentes. A avaliação imunológica incluiu a detecção dos níveis dos biomarcadores inflamatórios IL-1β, TNF-α e IL-6 através de ensaios de ELISA. Os dados foram submetidos à análise estatística considerando p≤ 0,05. Os resultados mostraram que a condição periodontal de pacientes diabéticos e não diabéticos foi similar. Quando considerados pacientes com gengivite (IG ≥ 2), todos os índices lipídicos avaliados foram maiores no grupo dos diabéticos, com diferença estatística para HDL, TRG e LT. A prevalência do "complexo verde", principalmente *Cs* e *Co* foi maior nos sítios periodontais de crianças diabéticas.

Bactérias do "complexo vermelho" foram detectadas em poucos sítios dos grupos DM e NDM. *Fn* e *Cr*, do "complexo laranja", foram frequentemente encontrados em ambos os grupos. Níveis dos biomarcadores IL-1-β, TNF-α e IL-6 foram similares no soro de DM e NDM. Houve correlação positiva entre as variáveis lipídicas e imunológicas avaliadas somente para o grupo diabético. Conclui-se que os perfis periodontal, microbiológico e imunológico avaliados neste estudo foram similares entre as crianças diabéticas e não diabéticas. Os parâmetros glicêmicos e lipídicos foram maiores nos pacientes diabéticos, mas mantiveram-se dentro da normalidade, demonstrando que controle metabólico é essencial para a manutenção da saúde periodontal.

Palavras chave: gengivite, crianças, diabetes melito, PCR, bactérias anaeróbias, citocinas, lipídeos.

ABSTRACT

Periodontal disease (PD) comprises a group of inflammatory infections that affects the periodontal tissues and may be triggered by multiple local and systemic factors. Individuals of all age groups are susceptible to develop this disease and gingivitis, reversible condition limited to the gingival tissues, is commonly seen in children and in the early adolescence period. The presence of a specific pathogenic microbiota is an essential factor to PD development. Systemic diseases, such as diabetes mellitus, can increase the development and progression of the disease, mainly due to exacerbated immunological response and changes in physiological parameters that contribute to tissue injury. The aim of this study was to compare clinical, microbiological and immunological profiles of type 1 diabetes mellitus children (DM) to non-diabetic control group (NDM). A total of twenty four DM children and twenty seven NDM controls were evaluated. Periodontal status was assessed using plaque index, gingival index and probing depth and general health status were determined using glycemic levels, HBA1c (glycosylated haemoglobin), HDL, LDL, triglycerides (TRG), total cholesterol (TC), VLDL and total lipids (TL), from blood samples. Polymerase chain reaction (PCR) was used for identification of the following bacteria: *Aggregatibacter actinomycetemcomitans* (Aa), *Campylobacter rectus* (Cr), *Capnocytophaga sputigena* (Cs), *Capnocytophaga ochracea* (Co), *Eikenella corrodens* (Ec), *Fusobacterium nucleatum* (Fn), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) e *Prevotella nigrescens* (Pn) from gingival crevicular fluid of deciduous and permanent teeth. Immunological evaluation was determined by means of detection of inflammatory biomarkers -1β, TNF-α and IL-6 using ELISA assays. Data were submitted to statistical analysis, considering p≤ 0.05. The results showed that periodontal status of diabetic and non-diabetic patients was similar. Considering patients with gingivitis (GI ≥ 2), all lipid parameters evaluated were highest in DM group, however, statistical difference was observed only for HDL, TRG and TL. The prevalence of "green complex", mainly *Cs* and *Co*, was definitely more prevalent in periodontal sites of DM children. Bacteria from "red complex" were detected in few sites of DM and NDM groups. From the "orange complex", *Fn* and *Cr* were frequently found in both groups. Similar levels of the serum biomarkers, IL-1-β, TNF-α and IL-6, were

detected in the serum of DM and NDM children. In conclusion, clinical, microbiological and immunological profiles evaluated in this study were similar between diabetic and non-diabetic children. Glycemic and lipid parameters were higher in diabetic patients, but remained within normal values, demonstrating that metabolic control is essential for maintaining periodontal health.

Key words: gingivitis, children, diabetes mellitus, PCR, anaerobic bacteria, cytokines, lipids.

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"A mente que se abre a uma nova ideia

jamais voltará ao seu tamanho original."

Albert Einstein

LISTA DE ABREVIATURAS E SIGLAS

Aa   -   *Aggregatibacter actinomycetemcomitans*

AGEs -   Advanced glycation endproducts

BMP -   Proteína morfogenética óssea (de *Bone morphogenetic protein*)

ceod -   Índice de dentes decíduos cariados, com extração indicada e obturados CPOS

         -    Índice de dentes permanentes cariados, perdidos e obturados

Cr   -   *Campylobacter rectus*

Co   -   *Capnocytophaga ochracea*

CP   -   Clinical profile

Cs   -   *Capnocytophaga sputigena*

CT   -   Colesterol total

DM   -   Diabéticos Tipo I

DP   -   Doença periodontal

Ec   -   *Eikenella corrodens*

Fn   -   *Fusobacterium nucleatum*

HBA1c-   Hemoglobina glicosilada

HDL -   Lipoproteína de alta densidade

IFN–γ -   Interferon gama

IG   -   Índice gengival

IGD -   Índice gengival para dentes decíduos

IGP -   Índice gengival para dentes permanentes

IL-1β -   Interleucina 1 beta

IL-6 -   Interleucina 6

IP   -   Índice de placa

IPD -   Índice de placa para dentes decíduos

IPP -   Índice de placa para dentes permanentes

LDL -   Lipoproteína de baixa densidade

LT   -   Lipídeos totais

NDM -   Normoglicêmicos

PCR	-	Reação em cadeia da polimerase
Pg	-	*Porphyromonas gingivalis*
PGE_2	-	Prostaglandina E_2
Pi	-	*Prevotella intermedia*
Pn	-	*Prevotella nigrescens*
PS	-	Profundidade de sondagem
Td	-	*Treponema denticola*
Tf	-	*Tannerella forsythia*
TL	-	Total Lipids
TGF-β	-	Fator de crescimento transformador beta (de *Transforming growth factor*)
TRG	-	Triglicérides
TNF-α	-	Fator de necrose tumoral alpha
VLDL	-	Lipoproteína de muito baixa densidade

LIST OF ABREVIATIONS AND ACRONYMS

Aa   -   *Aggregatibacter actinomycetemcomitans*

AGEs   -   Advanced glycation end products

dmf   -   Decayed, missing and filled index for deciduous teeth

DMF   -   Decayed, missing and filled index for deciduous teeth

CD   -   Cristiane Duque

Cr   -   *Campylobacter rectus*

Co   -   *Capnocytophaga ochracea*

Cs   -   *Capnocytophaga sputigena*

DM   -   Type 1 Diabetic children

Ec   -   *Eikenella corrodens*

Fn   -   *Fusobacterium nucleatum*

GACGC-   Gabriela A. C. G. Camargo

GCF   -   Gingival crevicular fluid

GI   -   Gingival index

GID   -   Gingival Index for deciduous teeth

GIP   -   Gingival Index for permanent teeth

HBA1c-   Glycosylated haemoglobin

HDL   -   High –Density Lipoprotein

ICC   -   Intraclass correlation coefficient

IL-1 β -   Interleukin 1 beta

IL-6   -   Interleukin 6

LDL   -   Low –Density Lipoprotein

LPS   -   Lipopolysaccharide

MP   -   Microbiological profile

NDM   -   Non-diabetic children

PCR   -   Polymerase chain reaction

PD   -   Probing depth

PDD   -   Probing Depth for deciduous teeth

PDP   -   Probing Depth for permanent teeth

Pg   -   *Porphyromonas gingivalis*

PI   -   Plaque index

PID   -   Plaque index for deciduous teeth

PIP   -   Plaque index for permanent teeth

PGE_2 -   Prostaglandina E_2

Pi   -   *Prevotella intermedia*

Pn   -   *Prevotella nigrescens*

TC   -   Total cholesterol

Td   -   *Treponema denticola*

Tf   -   *Tannerella forsythia*

TL   -   Total Lipids

TRG   -   Triglycerides

TNF-α   -   Tumor necrosis factor alpha

VLDL   -   Very Low-Density Lipoprotein

INTRODUÇÃO

A doença periodontal (DP) é uma doença inflamatória crônica do periodonto, de caráter progressivo, induzida por bactérias e que leva à destruição dos tecidos de suporte do dente – osso, ligamento periodontal e cemento (Quirino *et al.*, 2009; Darveau, 2010). Ela é considerada uma das principais causas de perda precoce dos dentes e representa um problema de saúde pública de ampla distribuição mundial (Ministério da Saúde, 2003). Segundo a classificação da American Academy of Periodontology (Armitage, 1999), a DP pode ser dividida em doenças gengivais e periodontites. As doenças gengivais são subdivididas em gengivite associada à presença de biofilme dental (gengivite crônica), gengivite modificada por fatores sistêmicos, medicamentos e nutricionais e a hiperplasia gengival. As periodontites são subdivididas em crônica e agressiva, sendo considerada localizada ou generalizada, dependendo da extensão da doença (Armitage, 1999). O tipo mais comum de doença periodontal que afeta crianças e adolescentes é a gengivite crônica, com prevalência variada, desde 12% até mais de 90% com algum grau de alteração gengival (Coutinho e Tostel, 1997*;* Novais *et al.,* 1997; Bossnjak *et al.,* 2003; Xavier *et al.*, 2007). Pelo seu estado inicial, a gengivite em crianças é frequentemente caracterizada pela inflamação gengival sem perda óssea ou de inserção detectável (Ulker *et al.*, 2008).

Praticamente todas as faixas etárias são susceptíveis ao desenvolvimento da DP, e se um processo patológico acomete o periodonto de uma criança, em longo prazo esse pode ter influência sobre o periodonto do adulto (Mackler e Crawford, 1973; Orbak *et al.*, 2008). Diversos estudos, tanto transversais quanto longitudinais, envolvendo crianças de diferentes idades, mostraram que a severidade e a prevalência de gengivite aumentam com a idade (Hugson *et al.*, 1981; Mattson e Goldberg, 1985; Cortelli *et al.*, 2008; Orbak *et al.*, 2008). Sendo que, as diferenças na composição do biofilme dental, no sistema de defesa imunológica, na morfologia dos tecidos gengivais e na erupção dentária associada ao nível de maturidade psicológica e de destreza manual são os principais fatores que determinam a presença da doença periodontal na infância (Matson, 1993).

Como o início da doença periodontal pode ser causado pelo acúmulo de bactérias patogênicas na região subgengival formando o biofilme dental, existe uma forte associação

entre certas espécies bacterianas e seus hospedeiros susceptíveis. Em particular, às bactérias *Tannerella forsythia* (*Tannerella forsythensis* ou antigamente *Bacteroides forsythus*), *Porphyromonas gingivalis* e *Treponema denticola*, denominadas de complexo vermelho (Socransky *et al.*, 1998), têm sido atribuído papel importante em várias formas de doença periodontal em adultos (Grossi e Zambom, 1994; Haffajee e Socransky, 1994; Grossi *et al.*, 1995; Zambon, 1996; Socransky *et al.*, 1998; Slots e Ting, 1999). *Campylobacter sp.*, *Prevotella intermedia/Prevotella nigrescens* , *Fusobacterium sp.*, *E. nodatum*, entre outros, pertencentes ao complexo laranja (Socransky *et al.*, 1998) e *Aggregatibacter actinomycetemcomitans*, também estão sendo relacionados à destruição periodontal avançada como um grupo secundário de periodontopátogenos (Haffajee e Socransky, 1994; Zambon, 1996; Sakamoto *et al.*, 2002; Haffajee *et al.,* 2008). Ainda permanecem dúvidas em relação ao período de vida em que esses microrganismos iniciam a colonização na cavidade bucal e o início do desenvolvimento da doença periodontal. Poucos estudos avaliaram a prevalência desses microrganismos em idade precoce, utilizando técnicas laboratoriais e moleculares avançadas. Cortelli *et al.* (2008, 2009) verificaram altos níveis de *Campylobacter rectus* associados com saúde periodontal e *Prevotella intermedia* com algum grau de inflamação gengival em crianças de 6 a 12 anos.

Em estudos da distribuição desses patógenos, PCR (reação em cadeia da polimerase) tem sido uma técnica altamente sensível para detectar e identificar bactérias em amostras biológicas e, diversos achados científicos em Periodontia têm sido relatados utilizando esse método (Watanabe e Frommel, 1993; 1996; Ashimoto *et al.*, 1996; Riggio *et al.*, 1996; Garcia *et al.*, 1998; Sakamoto *et al.*, 1999). A técnica diagnóstica do PCR pode detectar reduzidos níveis de bactérias orais e particularmente ser útil para determinar os estágios iniciais de colonização oral e a distribuição de espécies diferentes de patógenos (Ashimoto *et al.,* 1996; Sakamoto *et al.*,1999).

A patogênese da doença periodontal tem sido revisada por diversos autores (Kornman *et al.*, 1997; Page *et al.*, 1997; Kornman, 1999; Preshaw, 2008). As bactérias são consideradas fatores importantes para o desenvolvimento, mas não suficientes para causar a doença (Kornman *et al.*, 1997; Page *et al.*, 1997). Fatores do hospedeiro tais como hereditariedade e imunidade e fatores ambientais e sistêmicos são igualmente importantes

para a ocorrência e severidade da doença (Page *et al.*, 1997). Estudos têm demonstrado que alterações na microbiota apresentada no sulco gengival resultam na subregulação das respostas imuno-inflamatórias nos tecidos periodontais que é caracterizada pela excessiva produção de citocinas, prostanóides e enzimas. Altos níveis destes mediadores inflamatórios são responsáveis, pelo menos em parte, pela conversão de lesões de gengivite estabelecidas em estados mais avançados de degradação tecidual, levando a sinais clínicos e sintomas da doença periodontal (Heasman *et al.*, 1993; Kornman, 1999; Preshaw, 2008).

Em sítios doentes, o desafio microbiano claramente resulta na alteração dos mecanismos de defesa normais do periodonto (Darveau, 2010), onde componentes da placa dental microbiana ativam a resposta imune local do hospedeiro por meio da indução da infiltração de células inflamatórias, incluindo linfócitos, macrófagos e leucócitos polimorfonucleares (Ulker *et al.*, 2008). A ativação destas células leva a secreção de citocinas específicas, incluindo interferon gama (IFN– γ), fator de necrose tumoral alpha (TNF-α), fator de crescimento transformador beta (TGF-β, de *transforming growth factor*), proteína morfogenética óssea (BMP, de *bone morphogenetic protein*), interleucinas IL-1α, IL-1 β, IL-6, IL-10, IL-12, IL-15, PGE-2 (prostaglandina E2), entre outras, que interferem na atividade de outras células imuno-inflamatórias e de fibroblastos. Alguns desses fatores, em particular IL-1β, TNF-α e IL-6 foram associados com lesões periodontais, sendo fortemente implicadas na reabsorção óssea (Preshaw, 2008). Ejeil *et al.* (2003) observaram altos índices de IL-1β, TNF-α e IL-6 em tecidos gengivais com diferentes graus de inflamação correlacionando sua presença com a perda substancial de colágeno. Além disso, também foi verificado que o sinergismo entre algumas citocinas, como IL-1β e TNF-α, pode induzir a produção de prostanóides como PGE-2 e o aumento da inflamação tecidual (Yucel-Lindberg *et al.*, 1999). A maioria dos estudos clínicos realizado em crianças que avaliam a presença das citocinas inflamatórias está relacionada com a periodontite agressiva. Fine *et al.* (2009) verificaram a presença de 21 quimiocinas/citocinas na saliva de crianças com periodonto saudável e aquelas que apresentavam previamente altos níveis de *A. actinomycetencomitans* e risco de desenvolverem periodontite agressiva. IL-1β foi uma das citocinas mais relacionadas com a perda óssea, sendo que 19 delas nem puderam ser detectadas ou tiveram baixos níveis de detecção. Um dos poucos estudos que

relacionaram gengivite em crianças e a produção de citocinas foi realizado por Ulker *et al.* (2008). Os autores avaliaram os níveis de IL-1β e TNF-α na saliva e fluido crevicular gengival de crianças entre 11 e 16 anos com periodonto saudável ou com gengivite. Foi verificado que no fluido gengival os níveis de IL-1β e TNF-α foram mais altos em pacientes com gengivite e estes foram positivamente correlacionados com profundidade de sondagem e perda de inserção clínica. Portanto, pode-se concluir que os níveis dos mediadores inflamatórios podem representar biomarcadores ideais da atividade da doença (Ulker *et al.*, 2008).

Fatores sistêmicos também podem estar relacionados à patogênese da doença periodontal. O diabetes melito (DM) é uma doença caracterizada pela alteração na tolerância a glicose e deficiência na metabolização de lipídios e carboidratos (AAP, 2000), ocasionando a clássica tríade de sintomas: poliúria, polidipsia e polifagia (Soskolne e Klinger, 2001). A hiperglicemia se desenvolve devido à reduzida secreção e/ou ação da insulina (Mealey e Oates, 2006). Baseada nestas duas condições, o diabetes melito pode ser classificado em dois tipos: tipo 1, pacientes apresentam deficiência de insulina produzida pelas células β do pâncreas e tipo 2, resultante da deficiência na molécula de insulina ou alteração no receptor de superfície celular, ocasionando deficiência na função da insulina (AAP, 2000; Taylor 2001). Diabetes tipo 2 ocorre mais frequentemente em obesos e a glicemia pode ser controlada através da adequação da dieta e do peso (AAP, 2000; Kawamura *et al.*, 2001; Aren *et al.*, 2003).

Estudos epidemiológicos têm demonstrado que a presença do diabetes pode ser considerada um fator de risco para doença periodontal também na infância (Grossi *et al.*, 1994; Papapanou, 1996; Soskolne e Klinger, 2001; Mealey e Oates, 2006). Grupos específicos de pacientes, como os que possuem diabetes tipo 1, apresentam maior risco de desenvolver gengivite e tem uma maior prevalência quando comparados à população geral de crianças e adolescentes (Ryan *et al.*, 2003), o que pode ser comprovado pelos estudos de Bernick *et al.*, 1975 e Gislen *et al.*, 1980, que avaliaram a presença de sinais de DP em crianças diabéticas e chegaram a conclusão de que a inflamação gengival e a presença de sangramento era mais frequente no grupo de diabéticos quando comparados ao controle. Cianciola *et al.* (1982) analisaram a condição bucal de 208 adolescentes e relataram

prevalência de 9,8% de doença periodontal em diabéticos na faixa etária de 11 a 18 anos, comparado a 1.7% de DP no grupo controle sem diabetes. De acordo com Taylor *et al.* (2001) a presença de diabetes aumenta a prevalência, incidência e severidade da doença periodontal. Orbak *et al.* (2008) investigaram os efeitos do diabetes sobre a dentição e saúde bucal de crianças e adolescentes. Os autores observaram inflamação gengival em 69.7% das crianças na faixa etária de 5 a 9 anos e em 83.7% das crianças entre 10 a 14 anos portadoras de diabetes melito tipo 1. Os autores verificam um aumento gradativo no índice gengival e índice de placa de acordo com a faixa etária avaliada.

O diabetes melito pode ser um fator de risco para o desenvolvimento/progressão da doença periodontal por meio de mecanismos incompletamente elucidados, que incluem alteração da função neutrofílica, glicosilação e ligação cruzada das fibras de colágeno, defeito na secreção de fatores de crescimento e mediadores inflamatórios e a produção de espécies reativas de oxigênio (Pérez *et al.*, 2004; Verma e Bath, 2004; Struch *et al.*, 2008; Unür *et al.*, 2008). Embora esses efeitos sistêmicos estejam relacionados com a exposição glicêmica excessiva, fatores genéticos ou ambientais podem levar a complicações da diabetes como doenças cardiovasculares, retinopatias, nefropatias e até a própria doença periodontal. Os maiores fatores de risco são a hipertensão, ato de fumar, índice de massa corporal e desordens lipídicas. Em adolescentes, além desses fatores, existe o impacto da puberdade sobre a diabetes (Gallego *et al.*, 2007). Como a insulina e os hormônios sexuais competem pelos receptores nos tecidos periféricos e os músculos são os principais responsáveis pela ingestão de glicose periférica, tem sido sugerido que os hormônios esteróides induzem diretamente a redução da sensibilidade dos tecidos periféricos à ação da insulina, chamada de resistência da insulina. Isso leva à necessidade do maior uso de insulina nos adolescentes (Alonso e Gonzáles, 2008).

Outra alteração comum em crianças com diabetes tipo I é a disfunção do endotélio, que está diretamente relacionada com a duração da diabetes e os níveis de triglicérides e colesterol LDL (lipoproteína de baixa densidade) (Gallego *et al.*, 2007). Estudos têm mostrado associação entre altos níveis lipídicos no soro (colesterol total e LDL) e a presença de doença periodontal em pacientes diabéticos (Merchant *et al.* 2011) ou normoglicêmicos (Taleghani *et al.*, 2010; Fentoglu *et al.*, 2011). A hiperlipidemia, causada

pelo desequilíbrio no metabolismo dos lipídeos afetado diretamente pelo aumento dos níveis de glicose sanguínea, induz a produção de citocinas inflamatórias, principalmente IL-1β e TNF-α e a supressão de fatores de proteção como TGF-β, tanto no soro quanto no fluido crevicular gengival em pacientes diabéticos (Ryan *et al.*, 2003). Esse desequilíbrio entre elevadas quantidades de citocinas e redução de fatores de crescimento com função protetora pode interferir na habilidade de reparo e facilitar a destruição tecidual (Cutler *et al.*, 1999).

As citocinas também são consideradas na diabetes melito como biomarcadores pró-inflamatórios e são encontrados em níveis mais elevados em pacientes diabéticos. Salvi *et al.* (2010) encontraram alta concentração de IL-1β em pacientes diabéticos tipo 1 em comparação com indivíduos saudáveis. Também verificaram que espécies bacterianas do grupo laranja e altos índices de placa (biofilme dental) apresentaram superior correlação com os níveis de biomarcadores quando comparados a outros complexos bacterianos ou outras mensurações clínicas durante a gengivite experimental. Snell-Bergeon *et al.* (2010) realizaram um estudo caso-controle com 553 pacientes diabéticos tipo 1 e 215 saudáveis de 10 a 22 anos de idade e verificaram que a diabetes tipo I é caracterizada por excessiva inflamação, independente do controle glicêmico e lipídico. Mesmo em jovens com bom controle glicêmico, altos níveis de IL-6 e outros marcadores foram maiores no grupo diabético. Esses marcadores foram associados com o perfil lipídico e podem contribuir para acelerada aterosclerose em jovens portadores de diabetes tipo 1. De modo geral, sabe-se que é comum algum desequilíbrio na produção de citocinas em ambas as doenças periodontal e diabetes, especialmente IL-1β, IL-6 e TNF-α (Pérez *et al.*, 2004; Moreira *et al.*, 2007a; Moreira *et al.*, 2007b; Struch *et al.*, 2008; Raunio *et al.*, 2009; Xiao *et al.*, 2009), as quais estão envolvidas na resposta imune inata, nos processos inflamatórios de reparo e na ação contra microrganismos (Kumar *et al.*, 2005).

Os objetivos deste estudo foram comparar os perfis clínico (geral e bucal), microbiológico e imunológico de crianças de 7 a 13 anos, portadoras ou não de diabetes melito tipo I e verificar possíveis correlações entre esses parâmetros e a presença de gengivite.

CAPÍTULO 1

"Relationship between microbiological, lipid and immunological profiles and the presence of gingivitis in type 1 diabetes mellitus children"

Abstract

Background: Little information is available on periodontopathic bacterial colonization and the influence of systemic alterations, such as diabetes mellitus, in the development of gingivitis in childhood. The aim of this study was to evaluate the relationship between microbiological, lipid and immunological profiles and the presence of gingivitis in children with type 1 diabetes mellitus (DM) when compared to non-diabetic children (NDM).

Methods: A total of twenty four DM children and twenty seven NDM controls with similar age and gender were evaluated in this study. The periodontal status was assessed using plaque index, gingival index and probing depth. Glycemic (fasting glucose level, glycosylated hemoglobin) and lipid (HDL, LDL, and triglycerides - TRG, total cholesterol - TC, VLDL and total lipids - TL) profiles were determined for both diabetic and non-diabetic patients with gingivitis. Polymerase chain reaction (PCR) detection was used to determine the prevalence of *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Prevotella intermedia* (Pi), *Prevotella nigrescens* (Pn), *Fusobacterium nucleatum* (Fn), *Campylobacter rectus* (Cr), *Capnocytophaga ochracea* (Co), *Capnocytophaga sputigena* (Cs) and *Eikenella corrodens* (Ec). Blood samples were collected for IL-1-β, TNF-α and IL-6 analysis using ELISA kits. Statistical significance was established at 5%.

Results: Periodontal conditions of diabetic and non-diabetic patients were similar, without statistical differences in periodontal indices. When considering patients with gingivitis (GI ≥ 2), all lipid parameters evaluated were highest in the DM group; however, a statistical difference was observed only for HDL, TRG and TL. *Cs* and *Co* were definitely more prevalent in the periodontal sites of DM children. "Red complex" bacteria were detected in few sites of DM and NDM groups. The "orange complex" bacteria, *Fn* and *Cr*, were frequently found in both groups. Similar levels of the serum biomarkers, IL-1-β, TNF-α and IL-6, were detected in DM and NDM children.

Conclusions: Clinical, microbiological and immunological profiles evaluated in this study were similar between diabetic and non-diabetic children. Glycemic and lipid parameters were higher in diabetic patients, but remained within normal values, demonstrating that metabolic control is essential for maintaining periodontal health.

Key words: gingivitis, children, diabetes mellitus, PCR, cytokines, lipids.

Introduction

Periodontal disease comprises a group of conditions that affects gingiva, periodontal ligament, cementum, alveolar bone, and tissue structures that support the teeth[1]. The predominant form of periodontal disease in children and adolescents is gingivitis[2]. This disease is characterized by inflammation of the marginal gingiva without detectable loss of bone or connective tissue attachment. The initial clinical findings in gingivitis include redness and swelling of marginal gingiva, and bleeding upon probing. As the condition persists, tissues that were initially edematous may become more fibrotic[3]. There is no clear-cut age at which the gingival reaction to bacterial insult in children converts to that found in adults. However, there is a gradual increase in gingival activity from early childhood to adult age[4].

The etiology of periodontal disease is complex. The continuous accumulation of dental biofilm may result in an imbalance between pathogenic species and the host defense mechanisms, which may lead to gingival inflammation. Some bacterial species are recognized as putative periodontal pathogens[5,6]. In particular, *Tannerella forsythia* (*Tannerella forsythensis*), *Porphyromonas gingivalis* and *Treponema denticola,* known as "red complex" pathogens, have been indicated for playing important roles in various forms of periodontal diseases[7,6]. *Campylobacter sp.*, *Prevotella intermedia/Prevotella nigrescens*, *Fusobacterium sp.*, members of the "orange complex", are also related to periodontal breakdown as the secondary group of periodontal pathogens[6]. *A. actinomycetemcomitans* is frequently associated with early-onset periodontitis, an especially localized aggressive periodontitis[8]. There are few studies that have examined microbial colonization and gingival health during childhood. Cortelli et al.[9,10] detected high levels of *Campylobacter*

rectus associated with periodontal health and *Prevotella intermedia* with the presence of inflammation. Rotimi et al.[11] showed that, except for *P. gingivalis*, periodontopathogens such as *A. actinomycetencomitans*, *T. forsythia*, *P. intermedia* and *P. nigrescens* are relatively common findings in the oral cavity of children. Thus, the relationship between clinical parameters and the prevalence of several periodontal pathogens in children needs to be studied in greater detail.

The pathogenesis of periodontal disease has been revised by several researchers [12-14] and there is a consensus that, although bacteria are essential, they are insufficient for the disease to occur[12]. Microbial challenge in the subgingival plaque modulates the host immune-inflammatory response in the periodontal tissues[13,14]. Macrophages and polymorphonuclear leukocytes, in response to the chemo-attractant effect of bacterial toxins, such as lipopolysaccharide (LPS), are activated to produce important inflammatory mediators —notably, TNF-α, IL-1-β, IL-6, and other cytokines[15,16]. These mediators are responsible for periodontal breakdown, leading to the clinical signs and symptoms of disease[13,14]. A few studies evaluated the production of cytokines in children with gingivitis. Ulker et al.[17] found a correlation between high levels of IL-1β and TNF-α in gingival crevicular fluid of children and clinical signs of gingivitis.

Systemic inflammatory diseases, such as diabetes, alter the host environment, and are predicted to increase the patient's vulnerability to gingivitis due to changes in the inflammatory response to microbial challenges[18,19]. Type 1 diabetes mellitus is a chronic autoimmune disease in which the immune system selectively destroys insulin-producing beta-cells of the pancreas, resulting in hyperglycemia due to the lack of insulin secretion[20]. There are few clinical studies that have demonstrated that the presence of diabetes can be considered a risk factor for periodontal disease in childhood[21-23]. Orbak et al.[23] observed greater plaque, gingival and calculus indices scores in diabetic children when compared to non-diabetic children, which increased with age.

Individuals with diabetes mellitus have impaired neutrophil and macrophage functioning, altered collagen production, and exaggerated collagenase activity[18], perhaps leading to the patient's heightened inflammatory state, as interactions with advanced glycation endproducts (AGEs) have been shown to increase macrophage secretion of

proinflammatory mediators[18]. Salvi et al. [19] found a high concentration of IL-1β in type 1 diabetic patients when compared to healthy individuals. Those authors also verified a correlation between this inflammatory biomarker and some bacterial species belonging to the orange complex in diabetic children. One of the consequences of hyperglycemia over time is hyperlipidemia. Snell-Bergeon et al. [24] evaluated 553 patients with type I diabetes mellitus and 215 healthy patients aged 10 to 22 years old and observed that high levels of IL-6 and other biomarkers were associated with the lipid profile and may collaborate with systemic complications in diabetic individuals. These complications could increase the patient's risk for development of severe periodontal disease[22]. The objective of this study was to study the relationship between microbiological, lipid and immunological profiles and the presence of gingivitis in children with type 1 diabetes mellitus.

Materials and methods

Study population

The study protocol was approved by Antonio Pedro University Hospital's Committee of Research, Fluminense Federal University (protocol 057/2010) (ANEXO 1A/B). Children with Type 1 diabetes mellitus and non-diabetic children, aged 7 to 13 years, with mixed dentition, of both genders, and without distinction of race were selected for this study. Non-diabetic individuals were recruited from the Pediatric Dentistry Clinic at Faculty of Dentistry - Fluminense Federal University (Nova Friburgo – RJ, Brazil) and diabetic children from the database kindly provided by Nova Friburgo Diabetes Association (ADINF). The exclusion criteria used for subject recruitment[11] were: (1) antibiotic prophylaxis for dental treatment, (2) uncontrolled systemic diseases, (3) immunological compromise, (4) subjects who were wearing orthodontic devices, (5) subjects who had been undergoing periodontal treatment 12 months before the beginning of the study, (6) those who had been taking antibiotics within 6 months prior to the clinical examination, (7) those with extensive caries lesions, (8) subjects who were using an antiseptic solution during 3 months period and (9) smokers. Parents or legal guardians were informed of the study and signed an informed consent form (ANEXO 2) and completed an interview regarding the medical and dental histories of the children (ANEXO 3).

Clinical measurements

The following clinical parameters were measured: probing depth (PD), plaque index (PI)[25] and gingival index (GI)[26], by two previously calibrated examiners (CD and GACGC), using a periodontal probe (PCPUNC 15) (Hu-Friedy, Chicago, IL) at four sites (mesio-bucal, mid-buccal, disto-lingual, mid-lingual) per tooth. The following teeth were examined: all first permanent molars, all second deciduous molars, two upper permanent incisors and lower permanent incisors (ANEXO 4). Permanent teeth were fully erupted. The intra-examiner and inter-examiner agreement of the categorical variables (PI, GI) using the Kappa calculation, at tooth level, was 0.72 and 0.68, respectively. Reproducibility of continuous variables (PD) was 0.71 and 0.69, respectively, as examined by the intraclass correlation coefficient (ICC).

Intraoral samples collection

Before the intraoral collection procedures, cotton rolls were applied to prevent contamination of the sampling area with other oral fluids. The supragingival biofilm was gently removed using sterile cotton pellets and subgingival biofilm samples were collected using sterile paper points (Tanari #30, Tanariman Industrial Ltda., Manacapuru, AM, Brazil) which were inserted to the depth of the gingival sulci for 60 seconds. This procedure was performed for each of the four sites previously selected (mesio-bucal sulci of three permanent molars and one permanent incisor, selected randomly or mesio-bucal sulci of four deciduous molars) and the paper points of each subject were inserted in a microtube 1 ml containing TE solution (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) on ice. Pooled biofilms were separated according to dentition (permanent or deciduous) for each patient. The samples were stored at –80°C until the analyses.

Blood samples collection

Patients were asked to reduce the intake of fatty foods the night before collecting the blood samples. Blood samples were collected (10ml – 7ml to clinical analysis and 3ml to immunological analysis) by a specialized professional from the peripherical vein (cubital

fossa) of individuals who had an overnight fast. Samples were collected in vacuum collection tubes and sent to Raul Sertã Hospital Laboratory at Nova Friburgo/RJ for clinical analysis (fasting glucose levels (GL), glycosylated hemoglobin – (HbA1c), triglycerides (TRG), Total Cholesterol (TC), High –Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Very Low-Density Lipoprotein (VLDL) and Total Lipids (TL) using specific kits (Gold Analisa, Belo Horizonte/MG). One tube was centrifuged (3000 rpm/10 min) and the blood serum was carefully collected, aliquoted and frozen at -80°C for immunological analysis.

Bacterium-specific PCR

The subgingival samples were thawed, vortexed and centrifuged (10000rpm/10 min). After removal of the paper points and supernatant, samples were submitted to DNA extraction using a protocol described originally by Doyle & Doyle [27] with some modifications. Briefly, samples were submitted to a lysing solution (extraction buffer and proteinase K) and then purified using chloroform:isoamil-alcohol, followed by DNA precipitation using isopropanol and 70% ethanol. The DNA was re-suspended in TE buffer with 10 mg/mL RNAse. DNA were quantified in a spectrophotometer at 260nm (Genesys 10UV, Rochester, NY, USA) in order to obtain a standard concentration of 100ng/ml and stored at -20°C. Bacterial molecular identification was carried out by Polymerase Chain Reaction method using a thermal cycler (TPersonal, Biometra, Germany). The bacterium-specific primer sequences and PCR conditions[28-31] used in this study are presented in **Table 1**. PCR reactions were standardized for each primer using genomic DNA from strains of culture collections as a positive control and distilled water as a negative control. PCR amplifications were performed using 200uM of dNTPs, 2.5mM of MgCl2, 0.3uM of each primer, 1.25U of Taq DNA polymerase (Invitrogen, Brazil) and approximately 10ng of genomic DNA, to obtain a volume of 25ul. Thermal conditions of each primer was tested, following the initial pattern: DNA denaturation at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, primer hybridization at 55°C-62°C (depending on the primer) for 30 seconds, extension at 72°C for 1 minute and finalizing the reaction at 72°C for 7 minutes [11]. The PCR products were separated by electrophoresis in 2% agarose gels and Tris-borate-EDTA

running buffer. The DNA was stained with 0.5ug/ml ethidium bromide and visualized under UV illumination (Pharmacia LKB-MacroVue, San Gabriel, CA, USA). Each gel received a 100pb or 1 Kb DNA Ladder (Invitrogen, Brazil).

ELISA assays

IL-1-β, TNF-α and IL-6 levels were determined by ELISA kis (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. Serum samples were assayed at 1:10 dilutions and evaluated in duplicate. A standard curve was constructed using the standards provided in the kits, and the cytokine concentrations were calculated from the standard curve. Biomarker quantification was performing using a microplate reader (Molecular Devices, Programa Versa Max). Results were reported as pg/ml.

Statistical analysis

The statistical analysis was performed using SPSS Statistics 17.0 (IBM Inc., Chicago, IL, USA). The subject characteristics (gender, age, fasting glucose level, HBA1c level and dmf/DMF) were compared between DM and NDM using Student t test for quantitative variables and Mann Whitney for qualitative variables. Clinical parameters were compared between DM and NDM using Mann Whitney test, except for PS, which was submitted to Student t test. The percentage of sites with the tested bacteria and data from the questionnaire were compared between DM and NDM applying the Chi-square test. Immunological and lipid profiles were assessed using Student t test. Pearson correlation test were applied to find positive associations between periodontal status and other clinical parameters (lipid, microbiological and immunological profiles). Differences were considered significant when p ≤ 0.05.

Results

Clinical characteristics of study population

Table 2 shows the subject characteristics of this study. 27 non-diabetic children and 24 children with type 1 diabetes mellitus participated in this study. No significant difference was observed between DM and NDM groups, considering gender, age and caries level

evaluation (dmf/DMF). Fasting glucose and HBA1c levels were statistically different between the groups, always with the highest values for the DM patients. PI, GI indexes and PS measurements were determined for deciduous and permanent teeth separately. **Tables 3 and 4** show PI and GI data obtained from percentage of sites with each score for both conditions: presence of diabetes and dentition. Means (standard deviations) of PD were 1.41 (0.49) and 1.41 (0.5) for NDM groups, considering deciduous and permanent teeth, respectively. For the DM group, means (standard deviations) of PD were 1.34 (0.29) and 1.48 (0.5), considering deciduous and permanent teeth, respectively. There were no significant differences for the majority of PI and GI scores and all PD measurements when comparing DM and NDM for both dentitions, indicating that both groups had similar periodontal conditions. Interviews given to children and their parents provided information about the gingivitis history and dental care of patients, as showed in **Tables 5 and 6**, respectively. There were no differences between DM and NDM for the parameters evaluated in both tables.

For the next analysis, only children (22 non-diabetics and 21 diabetics) presenting at least one periodontal site for each dentition with GI ≥2, i.e., showing gingival bleeding, were considered in order to study the relationship between the presence of gingivitis and lipid, microbiological and immunological profiles.

Lipid profile

Table 7 presents the lipid profiles of DM and NDM groups, considering children with GI ≥2. All lipid parameters evaluated were highest in the DM group; however, a statistical difference was observed only for HDL, TRG and TL.

Microbiological profile

Tables 8 and 9 show relative and absolute frequencies of periodontal bacteria, as well as each species combination, detected in crevicular gingival fluid of DM and NDM children, considering deciduous and permanent teeth and GI ≥2. The DM group presented

statistically higher levels of *Capnocytophaga sputigena* for both dentitions and *Capnocytophaga ochracea* for permanent dentition when compared to the NDM group. *Prevotella intermedia* was detected in only two DM patients and *Aggregatibacter actinomycetemcomitans* was not detected in any children in this study. *Fusobacterium nucleatum* and *Campylobacter rectus* were the most prevalent bacteria, followed by *Eikenella corrodens*, in both populations. Bacteria from the "red complex" were detected in few sites of both the DM and NDM groups. The best combination of "orange complex" pathogens was *Fusobacterium nucleatum* and *Campylobacter rectus*. However, both of them combined with *Prevotella nigrescens* harbored around 42% and 50% of sites of NDM and DM groups, respectively. The "green complex", represented by the combination of *Eikenella corrodens*, *Capnocytophaga sputigena* and *Capnocytophaga ochracea* in this study, was definitely more prevalent in the periodontal sites of DM children. Even with the inclusion of *Campylobacter rectus* in the "green complex", this result did not change.

Immunological profile

Means and standard deviation values obtained for IL-1-β, TNF-α and IL-6 detected in the serum of children are shown in **Table 10**. There were no statistical differences between DM and NDM for any evaluated parameters, when considering patients with GI ≥2.

Association between periodontal status and lipid/microbiological and immunological profiles

Tables 11 and 12 present positive correlations found among some variables evaluated in this study. When comparing the periodontal status and cytokine levels, TNF-α and IL-6 was correlated with plaque accumulation (PID, PIT) in diabetic patients. When considering the immunological and lipid profiles, IL-1β was positively correlated with LDL, TC and TL for diabetic children and with TRG for non-diabetic children (p≤0.05).

Discussion

Periodontal disease is an infectious disease caused by the interaction between microorganisms, their products and the acerbated host immune response, resulting in destruction of the tooth-supporting tissues and periodontal ligaments [32]. Although the severity of this disease is less intense in children than in adults, persistent gingival inflammation could develop severe forms of periodontitis over the years [33].

In the present study, the following clinical criteria were evaluated: presence of dental plaque, gingival inflammation and probing depths using standardized periodontal indexes. The majority of the children, regardless of the presence of diabetes mellitus, showed good gingival health, which is demonstrated by the low percentage of scores above 2 and reduced probing depths (average of approximately 1.5 mm), even with moderate biofilm accumulation, mainly in the permanent dentition. These results are in accordance with studies performed with normoglycemic children[9-11]. In contrast, most studies with diabetic children detected higher levels of gingival inflammation when compared to non-diabetic patients, suggesting that diabetes is an aggravating factor for periodontal disease[34-36]. The current clinical results are in agreement with data obtained by Sbordone *et al.* [37,38]. Those authors observed that, even after 3 years of monitoring, no differences where detected in the periodontal evaluation of type 1 diabetics when compared to non-diabetic patients[38]. When comparing periodontal studies, differences may be detected in the clinical examinations related to recording design, type/number of sites assessed and the periodontal probe used to measure PD[39]. The protocol used in this study (four periodontal sites) showed the smallest bias and highest sensitivity of prevalence estimates amongst other tested protocols, according to Susin et al.[40]. Additionally, good oral hygiene habits, as present in this study, helped to maintain the periodontal health of both groups. Other important factors that could also have influenced the clinical results of different studies are the duration of diabetes and the glucose levels[41,36]. Al-Khabbaz et al.[36] observed that, in the type 1 diabetic children, periodontitis was significantly associated with longer duration of diabetes and older age at diagnosis of diabetes. Studies have demonstrated that poor metabolic control, including high levels of fasting glucose and glycosylated hemoglobin are important factors

that could increase the susceptibility to periodontal disease, as well as other systemic complications of diabetes mellitus[42].

One of the consequences of high glucose levels over time is hyperlipidemia, which induces the production of inflammatory cytokines and the suppression of host protective factors, such as TGF-β. This imbalance interferes in the tissue repair mechanisms and consequently increases tissue destruction in diabetic patients[43]. Snell-Bergeon et al. [24] evaluated 553 patients with type I diabetes mellitus and 215 healthy patients, aged 10 to 22 years old, and observed that high levels of IL-6 and other biomarkers were associated with lipid profile and may collaborate with systemic complications in diabetic individuals. The same was observed in our study. IL-6 was positively correlated with LDL, TC and TL for both groups. Although most of the lipid parameters were higher in diabetic children when compared to non-diabetic children, statistical differences were observed only for HDL, TRG and TL. It is possible that these differences were observed with the increase of the individuals in this study. Lim et al. [44] evaluated one hundred and eighty one adult patients with diabetes and studied the relationship between metabolic control markers and inflammation on the severity of periodontal disease in patients with diabetes mellitus. Those authors found positive correlations between HbA1c and the percentage of sites with probing depths > or = 5 mm, total cholesterol, LDL and triglycerides. Studies evaluating lipid parameters and periodontal disease in diabetic children or adolescents were not found.

The presence of putative periodontal pathogens was evaluated using PCR and specific primers to *Aggregatibacter actinomycetemcomitans* (Aa), *Campylobacter rectus* (Cr), *Capnocytophaga sputigena* (Cs), *Capnocytophaga ochracea* (Co), *Eikenella corrodens* (Ec), *Fusobacterium nucleatum* (Fn), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi) and *Prevotella nigrescens* (Pn). Most of these bacteria (mainly those from the "red complex": *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola*) have been related to the pathogenesis of periodontal disease and are frequently found in patients with chronic periodontitis[7,45,46]. In this present study, the prevalence of bacteria from the "red complex" was low and there was no difference between DM and NDM groups. When considering the "orange complex", *P. intermedia* was detected only in the permanent teeth

of two patients. Fn and Pn were detected in about half of the evaluated sites on both dentitions for both DM and NDM subjects. One interesting result was the marked combination of Fn and Pn in the permanent dentition. Okuda et al.[47] observed synergism between *Prevotella* species, including Pn, and *Fusobacterium nucleatum*, in biofilm formation, suggesting that these Gram-negative bacteria in the subgingival crevice could play an important role in the development of chronic periodontitis. Although *Campylobacter rectus* has been included in the "orange complex", which is considered a secondary group of periodontal pathogens[6], some studies have demonstrated the presence of these species with gingival health[9,10]. In this current study, the "green complex", represented by the combination of *Eikenella corrodens*, *Capnocytophaga sputigena* and *Capnocytophaga ochracea*, was definitely more prevalent in the periodontal sites of children with DM. Kimura et al.[48] observed that some putative periodontal bacteria, such as *E. corrodens, A. actinomycetencomitans, C. sputigena, C. ochracea* and *C. rectus,* colonize earlier in the oral cavity than other species also related to periodontal disease. Unfortunately, that study did not include diabetic individuals. A few studies have evaluated the microbiota from subgingival sites of children with type 1 diabetes mellitus and no consistent data regarding the relationship between periodontal pathogens and diabetes were found[19,38]. A recent study showed that subjects with type 2 diabetes subjects with chronic periodontitis presented higher percentages of *Aggregatibacter, Neisseria, Gemella, Eikenella, Selenomonas, Actinomyces, Capnocytophaga, Fusobacterium, Veillonella and Streptococcus* genera, and lower percentages of *Porphyromonas, Filifactor, Eubacterium, Synergistetes, Tannerella* and *Treponema* genera when compared to non-diabetic individuals. Moreover, some phylotypes, such as *Fusobacterium nucleatum, Veillonella parvula*, *V. dispar* and *Eikenella corrodens* were detected significantly more often in diabetic subjects than in non-diabetic subjects[49].

The inflammatory host response to an oral bacterial challenge is a critical determinant in the outcome of patient health or disease. Systemic inflammatory diseases, such as diabetes, alter the host environment and could increase the patient's vulnerability to gingivitis due to changes in the inflammatory response to microbial challenges[18,19,22]. Cytokines are considered good biomarkers for periodontitis and other systemic

inflammatory diseases [50]. Gingival crevicular fluid (GCF) is considered a good source of locally and systemically derived biomarkers of periodontal disease. However, the collection of GCF in children is difficult because the gingival sulci in the primary teeth are shallower than in permanent teeth and this biological fluid is produced at a low rate. In this present study, the immunological analyses did not show differences between IL-1β, TNF- α and IL-6 levels among diabetic and non-diabetic children. However, a positive correlation between these serum biomarkers (IL-1 for NDM and TNF- α and IL-6 for DM) and gingival status was observed. Ulker *et al.*[17] observed higher levels of IL-1β and TNF-α in the gingival fluid of patients who had gingivitis when compared to those with a healthy periodontium. Those findings were positively correlated with probing depths and clinical attachment loss. Salvi et al.[19] found a high concentration of IL-1β and IL-8 in type 1 diabetic patients when compared to healthy individuals in an experimental gingivitis method. Those authors also verified a correlation between this inflammatory biomarker and some bacterial species belonging to the "orange complex" in diabetic children.

In conclusion, the clinical, microbiological and immunological profiles evaluated in this current study were similar between diabetic and non-diabetic children. Glycemic and lipid parameters were higher in diabetic patients, but remained within normal values. Good metabolic control is definitely important in preventing periodontal complications in young patients with diabetes, similarly to what is well established for other systemic complications of gingivitis. Longitudinal clinical studies using larger patient groups are necessary to confirm the influence of diabetes on the microbiological and immunological parameters and their relationship with gingivitis in children.

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Table 1 – Primers and PCR conditions

Primers (sequence 5'- 3' F/R)	Size (bp)	Annealing temperature (°C)	Reference
Aggregatibacter actinomycetemcomitans (Aa) AGAGTTTGATCCTGGCTCAG CACTTAAAGGTCCGCCTACGTGCC	593	60	Conrads *et al.*, 1996
Campylobacter rectus (Cr) TTTCGGAGCGTAAACTCCTTTTC TTTCTGCAAGCAGACACTCTT	598	60	Ashimoto *et al.*, 1996
Capnocytophaga ochracea (Co) AGAGTTTGATCCTGGCTCAG GATGCCGTCCCTATATACGGGG	185	55	Conrads et al, 1996
Capnocytophaga sputigena (Cs) AGAGTTTGATCCTGGCTCAG GATGCCGCTCCTATATACCATTAGG	185	55	Conrads *et al.*, 1996
Eikenella corrodens (Ec) CTACTAAGCAATCAAGTTGCCC CTAATACCGCATACGTCCTAAG	688	60	Ashimoto *et al.*, 1996
Fusobacterium nucleatum (Fn) CGCAGAAGGTGAAAGTCCTGTAT TGGTCCTCACTGATTCACACAGA	101	65	Suzuki *et al.*, 2004
Tannerella forsythia (Tf) *GCGTATGTAACCTGCCCGCA* *TGCTTCAGTGTCAGTTATACCT*	641	60	Ashimoto *et al.*, 1996
Treponema denticola (Td) CTAATACCGCATACGTCCTAAG CTAATACCGCATACGTCCTAAG	311	55	Watanabe *et al.*, 1996
Porphyromonas gingivalis (Pg) AGGCAGCTTGCCATACTGCGG ACTGTTAGCAACTACCGATGT	404	60	Cortelli *et al.*, 2008
Prevotella intermedia (Pi) *TTTGTTGGGGAGTAAAGCGGG* *TCAACATCTCTGTATCCTGCGT*	575	55	Ashimoto *et al.*, 1996
Prevotella nigrescens (Pn)	804	55	Ashimoto *et al.*, 1996

		ATGAAACAAAGGTTTTCCGGTAAG		
		CCCACGTCTCTGTGGGCTGCGA		
		Universal primer		
		AGAGTTTGATCCTGGCTCAG	348	55	Conrads *et al.*, 1996
		GGCTACCTTGTTACGACTT		

Table 2 – Characteristics of study population (NDM – non-diabetics / DM – diabetics)

Variables		NDM	DM
Gender- N	Male	13 (48.1)	12 (50)
(%)	Female	14 (51.9)	12 (50)
Age in years - Mean (SD)		9.62 (1.86)	9.45 (1.69)
Fasting glucose level mg/dl - Mean (SD)		78.7 (8.10)*	101.74 (40.64)
HbA1c % mg/dl - Mean (SD)		4.42 (0.61)*	6.94 (1.58)
Number of children (%) using insulin for more than 1 year		-	13 (54.16)
dmf/DFM		0.93/0.11	0.94/0.13

*Statistical difference when compared NDM vs. DM, according to Student's t test for quantitative data and Mann Whitney for qualitative data (p ≤0.05).

Reference values for children:

Fasting glucose level: from 70 to 99 (diabetics: above 140).

Glycated hemoglobin (HbA1c): from 4.5 to 7.0.

Table 3 – Percentage of scores obtained for the plaque index (PI - Silness & Löe[25]), considering NDM and DM groups.

Scores		0	1	2	3	> 1	> 2
PID							
NDM	Mean	27.8	40.4*	15.4	16.4	72.2	31.82
	Median (25-75)	0 (0-54.7)	40 (9.3-70.3)	10.4 (0-26.5)	0 (0-10.9)	100 (45.31-100)	28.1 (6.25-45.3)
	Range	100	93.7	50	100	100	100
DM	Mean	26.7	60.7	6.5	6.1	73.3	10.9
	Median (25-75)	0 (0-56.25)	62.5 (37-90.6)	0 (0-8.3)	0 (0-7.8)	100(43-100)	0 (0-25)
	Range	100	100	31.2	58.3	100	58.3
PIP							
NDM	Mean	23.6	45.8	17.8	12.8	76.4	30.7
	Median (25-75)	6.3(0-28.1)	53.5(18.8-71.9)	9.37(0-25)	0 (0-18.8)	93.8 (71.9-100)	19.4 (6.25-46.7)
	Range	100	84.4	65.6	93.8	100	100
DM	Mean	28.7	45.6	7.6	18.1	71.3	25.8
	Median (25-75)	28.1 (0-49.2)	41.7 (28-71.8)	3.1 (0-12.5)	6.3 (0-33.3)	71.8 (50–100)	25 (3.9-32.8)
	Range	96.8	100	28.1	100	100	100

PID = Plaque index for deciduous teeth.
PIP = Plaque index for permanent teeth.
*Statistical difference when compared NDM vs. DM, according to Mann Whitney test (p ≤0.05).

Table 4 – Percentage of scores obtained for Gingival Index (GI – Löe &Silness[26]), considering NDM and DM groups.

Scores		0	1	2	3	> 1	> 2
GID							
NDM	Mean	59.6*	18.4	20.9	1.0	40.4	21.9
	Median (25-75)	78.1 (18.75-100)	12.5 (0-31.3)	4.2 (0-37.5)	0 (0-0)	21.9 (0-81.3)	4.16 (0-43.8)
	Range	100	81.3	100	6.25	100	100
DM	Mean	26..7	60.7	6.5	6.1	73.3	10.9
	Median (25-75)	0 (0-34.4)	62.5 (0-12.5)	0 (0-21.9)	0 (0-0)	100 (0-66.7)	0 (0-12.5)
	Range	100	93.8	100	33	100	100
GIP							
NDM	Mean	48.9	30.3*	17.6	2.9	50.8*	20.4
	Median (25-75)	40.6 (12.5 -83.3)	21.9 (35.7 – 60.7)	9.37 (0-28.1)	0 (0-0)	59.4 (16.7 -87.5)	12.5 (6.25 – 28.1)
	Range	100	81.3	68.8	68.8	100	81.3
DM	Mean	69.7	12.7	15.3	2.3	30.3	17.6
	Median (25-75)	81.3 (39.1-96.1)	3.1 (0 -25)	6.3 (3.1-25)	0 (0-0)	18.8 (3.1-56.3)	8.3 (3.1-25)
	Range	100	75	100	25	100	100

GID = Gingival Index for deciduous teeth.
GIP = Gingival Index for permanent teeth.
*Statistical difference when compared NDM vs. DM, according to Mann Whitney test (p ≤0.05).

Table 5 – Gingivitis history.

Questions#		NDM n (%)	DM n (%)	Total n (%)
Gingival bleeding	Yes	10 (37)	6 (25)	16 (31.4)
Gingival pain	Yes	4 (14.8)	4 (16.7)	8 (15.7)
Halitosis	Yes	13 (48.1)	8 (33.3)	21 (41.2)
Bad taste on the mouth	Yes	7 (25.9)	8 (34.8)	15 (30)
Mouth breathing	Yes	16 (59.3)	10 (43.5)	26 (52)

#Note: There was no statistical difference for any of evaluated parameters – Chi square (χ^2) test

Table 6 – Oral hygiene habits.

Questions[#]		NDM n (%)	DM n(%)	Total N (%)
Tooth brushing	Yes	27 (100)	24 (100)	51 (100)
How many times brush the teeth?	3 or more	25 (92.6)	18 (75.0)	43 (83.3)
Who brushes the child teeth?	The own child	18(85.7)	20 (95.2)	38 (90.5)
	helped by his/her mother	3 (14.3)	1 (4.8)	4(8,2)
Bristle type of brush	Soft	22 (81.5)	14(73.7)	36 (78.3)
Use of mouthrinses	Yes	12 (44.4)	4 (18.2)	16 (32.7)
Use of dental floss	Yes	12 (44.4)	10 (45.5)	22 (44.9)
Use of topic fluoride	Yes	18 (66.7)	14 (58.3)	32 (62.7)
Topic fluoride in school	Yes	5 (20)	5 (22.7)	10 (21.3)
Topic fluoride in dentist	Yes	12 (46.2)	12 (54.5)	24 (50)

[#]Note: There was no statistical difference for any of evaluated parameters – Chi square (χ^2) test.

Table 7 – Mean (standard deviation) of values obtained for glycemic and lipid profile of children with gingival bleeding (GI>2), considering the NDM and DM groups (values in mg/dl).

	HDL	LDL	TRG	TC	VLDL	TL
GI>2						
NDM	45.9 (8.4)*	82.7(21.5)	66.9(27.0)*	142.5(27.7)	13.5 (5.6)	389.0(78.1)*
DM	54.4(15.5)	106.4 (37.5)	78.9(53.3)	167.5(46.5)	16.12 (10.4)	490(143.5)

HDL – high density lipoprotein, LDL – low density lipoprotein, TRG – triglycerides, TC – total cholesterol, VLDL – very low density lipoprotein, TL – total lipids.

* Statistical difference when compared NDM vs. DM, according to Student's t test (p ≤0.05).

Reference values for children (mg/dl)

HDL: desirable: above 45, limitrophe: 35 to 45, undesirable: below 45.

LDL: desirable: below 130, limitrophe: between 130 and 150, high: above 150.

TRG: from 10 to 150

TC: desirable: below 200, limitrophe: 200 a 239, elevated: above 240.

VLDL: until 30

TL: from 400 to 800.

Table 8 - Absolute and relative frequency values – n (%) – of bacteria detected on crevicular gingival fluid of children with gingival bleeding (GI ≥ 2), considering deciduous and permanent teeth.

	Deciduous teeth		Permanent teeth		Total	
	NDM (13 sites)	DM (17 sites)	NDM (22 sites)	DM (20 sites)	NDM(35 sites)	DM (37 sites)
Aggregatibacter actinomycetemcomitans (Aa)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Porphyromonas gingivalis(Pg)	4 (30.8)	5 (29.4)	10 (45.5)	7 (35)	14 (40)	12 (32.4)
Tannerella forsythia (Tf)	3(23.1)	7 (41.2)	11 (50)	8 (40)	14 (40)	15 (40.5)
Treponema denticola (Td)	0 (0)	2(11.8)	2 (9.5)	3 (15)	2 (5.7)	5 (13.5)
Prevotella intermedia (Pi)	0 (0)	0 (0)	0 (0)	2 (10)	0 (0)	2 (5.4)
Prevotella nigrescens (Pn)	15 (38.5)	9 (52.9)	10 (47.6)	10 (50)	25 (71.4)	19 (51.4)
Fusobacterium nucleatum (Fn)	13 (100)	17(100)	19 (86.4)	20 (100)	32 (91.4)	37 (100)
Eikenella corrodens (Ec)	13 (100)	17(100)	20 (90.9)	18 (90)	31(88.5)	35 (94.5)
Capnocytophaga sputigena (Cs)	0(0)*	11 (64.7)	2 (9.1)*	13 (65)	2 (5.7)*	24(64.9)
Capnocytophaga ochracea (Co)	4(30.8)	9(52.9)	5(23.8)*	12(63.2)	9(25.7)*	21(56.8)
Campylobacter rectus (Cr)	11(84.6)	15(88.2)	21 (95.5)	19 (95)	32 (91.4)	34 (91.9)

* Significant difference between DM and NDM, according to Chi square (χ^2) test. (p ≤0.05).

Table 9 - Absolute and relative frequency values – n (%) – of bacteria combinations detected on crevicular gingival fluid of children with gingival bleeding (GI ≥ 2), considering deciduous and permanent teeth.

	Deciduous teeth		Permanent teeth		Total	
	NDM (13 sites)	DM (17 sites)	NDM (22 sites)	DM (20 sites)	NDM(35 sites)	DM (37 sites)
Red complex						
Pg+Tf	1(7.7)	2 (11.8)	5 (22.7)	5 (25)	6 (17.2)	7 (18.9)
Pg+Td	0	0	0	1 (5)	0	1 (2.7)
Tf+Td	0	2 (11.8)	1(4.5)	2 (10)	1 (2.8)	4 (10.8)
Pg+Tf+Td	0	0	0	1 (5)	0	1 (2.7)
Orange complex						
Pi+Fn	0	0	0	2 (10)	0	2 (5.8)
Pi+Pn	0	0	0	0	0	0
Pi+Cr	0	0	0	2	0	2 (5.8)
Fn+Pn	5 (38.4)	9 (52.9)	10 (45.5)	10 (50)	15(42.8)	19 (51.4)
Fn +Cr	11 (84.6)	15 (88.2)	21 (95.5)	19(95	32 (91.4)	34 (91.9)
Cr+Pn	5 (38.4)	8 (47.1)	10(45.5)	10 (50)	15 (42.9)	18 (48.6)
Pi+Fn+Pn	0	0	0	0	0	0
Pi+Fn+Pn+Cr	0	0	0	0	0	0
Green complex						
Ec+Cs	0*	11 (64.7)	2 (9)*	10 (50)	2 (5.7)*	21 (56.8)
Ec+Co	4 (30.7)	9 (52.9)	4 (18.1)*	10 (50)	8 (22.9)*	19 (51.4)
Cs+Co	0*	9 (52.9)	1 (4.5)*	9 (45)	1 (2.8)*	18 (48.6)
Ec+Cs+Co	0*	9 (52.9)	1 (4.5)*	7 (35)	1 (2.8)*	16 (43.2)
Green complex (+Cr)						
Ec+Cr	11 (84.6)	15 (88.2)	21 (95.5)	17 (85)	32 (91.4)	32 (86.5)
Co+Cr	4 (30.7)	7 (41.1)	4 (18.1)*	12 (60)	8 (22.9)*	19 (51.4)
Cs+Cr	0*	9 (52.9)	2 (9)*	13 (65)	2 (5.4)*	22 (59.5)
Ec+Cs+Cr	0*	9 (52.9)	2 (9)*	11 (55)	2 (5.4)*	20 (54.0)
Ec+Co+Cr	4 (30.7)	7 (41.1)	2 (9)*	10 (50)	6 (17.1)*	17 (45.9)
Cs+Co+Cr	0*	7 (41.1)	1 (4.5)*	9 (45)	1 (2.8)*	16 (43.2)
(Ec+Cs+Co)+ Cr	0*	7(41.1)	1 (4.5)*	7 (35)	1 (2.8)*	14 (37.8)

* Significant difference between DM and NDM, according to Chi square (χ^2) test. (p ≤0.05).

Table 10 - Means (SD) of immunologic profile obtained for children with gingival bleeding (GI≥2), considering NDM and DM groups.

	IL-1β	TNF-α	IL-6
NDM	1.98 (0.29)	15.01 (2.91)	2.18 (0.29)
DM	1.57 (0.31)	11.48 (5.73)	1.26 (0.30)

Note1: There was no statistical difference when compared NDM vs. DM, according to Student's t test ($p \leq 0.05$).

Note 2: There was positive correlation between TNF-α and IL-6 for DM group, according to Pearson Correlation ($p \leq 0.01$)

Table 11 - Associations between clinical (PI, GI and PD) and immunological results for children with gingival bleeding (GI ≥ 2), considering NDM and DM groups.

	Condition	PID	PIP	PIT	GID	GIP	GIT	PDD	PDP	PDT
1L-1β	**NDM**	r=-0.2368	r=-0.1314	r=-0.0873	r=0.3629	r=0.2810	r=0.3969	r=0.1170	r=0.5629	r=0.3910
		p=0.459	p=0.684	p=0.787	p=0.246	p=0.376	p=0.201	p=0.717	p=0.057	p=0.209
	DM	r=0.4102	r=0.5387	r=0.5664	r=0.2995	r=0.2363	r=0.2847	r=-0.1141	r=0.2819	r=0.0619
		p=0.361	p=0.212	p=0.185	p=0.514	p=0.610	p=0.536	p=0.807	p=0.540	p=0.895
TNF-α	**NDM**	r=0.3277	r=0.0482	r=0.3740	r=0.0010	r=-0.5255	r=-0.3758	r=-0.2531	r=-0.7000	r=-0.5589
		p=0.298	p=0.882	p=0.231	p=0.998	p=0.079	p=0.229	p=0.427	p=0.011*	p=0.059
	DM	r=0.9346	r=0.6450	r=0.8011	r=0.7719	r=0.3044	r=0.5176	r=0.0570	r=0.0382	r=0.0523
		p=0.002*	p=0.118	p=0.030*	p=0.042*	p=0.507	p=0.234	p=0.903	p=0.935	p=0.911
IL-6	**NDM**	r=-0.2206	r=-0.2787	r=-0.3017	r=-0.1404	r=-0.1070	r=-0.1654	r=-0.2270	r=-0.3676	r=-0.3570
		p=0.491	p=0.380	p=0.341	p=0.663	p=0.741	p=0.607	p=0.478	p=0.240	p=0.255
	DM	r=0.9227	r=0.6076	r=0.7789	r=0.5371	r=0.0501	r=0.2591	r=0.0313	r=-0.0642	r=-0.0109
		p=0.003*	p=0.148	p=0.039*	p=0.214	p=0.915	p=0.575	p=0.947	p=0.891	p=0.982

PID = Plaque index for deciduous teeth, PIP = Plaque index for permanent teeth, PIT = Plaque index for all teeth, GID = Gingival index for deciduous teeth, GIP = Gingival index for permanent teeth, GIT=Gingival index for all teeth. PDD = probing depth for deciduous teeth, PDP= probing depth for permanent teeth, PDT=probing depth for all teeth.

* Positive correlation obtained by Pearson correlation analysis (sig. 1-tailed) - p≤0.05

Table 12 - Associations between lipid and immunological results for children with gingival bleeding (GI ≥ 2), considering NDM and DM groups.

	Condition	glucose	HBA1c	HDL	LDL	TRG	TC	VLDL	TL
IL-1β	NDM	r=0.2701 p=0.236	r=0.1919 p=0.405	r=0.0079 p=0.973	r=-0.2876 p=0.206	r=0.4436 p=0.044*	r=-0.1416 p=0.540	r=0.4119 p=0.064	r=0.0333 p=0.886
	DM	r=00.5333 p=0.139	r=0.2961 p=0.439	0.2561 p=0.506	r=0.6958 p=0.037*	r=0.1580 p=0.685	r=0.6710 p=0.048*	r=0.1815 p=0.640	r=0.7181 p=0.029*
TNF-α	NDM	r=-0.0856 p=0.712	r=-0.2661 p=0.244	r=0.0426 p=0.855	r=0.2157 p=0.348	r=-0.0142 p=0.951	r=0.1473 p=0.524	r=-0.1148 p=0.620	r=0.0908 p=0.695
	DM	0.3050 p=0.425	0.1283 p=0.742	0.5249 p=0.147	-0.0419 p=0.915	r=-0.3790 p=0.314	r=0.0818 p=0.834	r=-0.3780 p=0.316	r=-0.1065 p=0.785
IL-6	NDM	r=-0.1447 p=0.532	r=-0.1661 p=0.472	r=0.0833 p=0.720	r=0.5008 p=0.021*	r=0.1670 p=0.469	r=0.4177 p=0.060	r=0.0661 p=0.776	r=0.3702 p=0.099
	DM	r=0.1604 p=0.680	r=0.0771 p=0.844	r=0.7914 p=0.011*	0.2441 p=0.527	r=-0.3838 p=0.308	r=0.3987 p=0.288	r=-0.3738 p=0.322	r=0.1642 p=0.673

* Positive correlation obtained by Pearson correlation analysis (sig. 1-tailed) - p≤0.05

CONCLUSÃO

- Os índices periodontais (IP, IG e PS) são similares entre os grupos diabéticos e não diabéticos.

- *Capnocytophaga* é o gênero de maior prevalência nos sítios periodontais de crianças diabéticas.

- Pacientes com gengivite (IG $\geq$ 2), apresentam os índices lipídicos maiores em diabéticos para HDL, TRG e LT.

- Níveis dos biomarcadores IL-1-β, TNF-α e IL-6 são similares no soro de crianças diabéticas e não diabéticas.

- Há correlação positiva entre as variáveis lipídicas e imunológicas avaliadas somente para o grupo diabético.

- Os perfis clínico (geral e bucal), microbiológico e imunológico avaliados neste estudo são similares entre as crianças diabéticas e não diabéticas.

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[*] De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Comittee of Medical Journal Editors – Grupo de Vancouver. Abreviaturas dos periódicos de acordo com o Medline.

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ANEXO

ANEXO 1 (A) - Documento de aprovação do projeto pelo Comitê de Ética em Pesquisa.

UNIVERSIDADE FEDERAL FLUMINENSE

Comitê de Ética em Pesquisa da Faculdade de Medicina / Hospital Universitário Antônio Pedro

Herbert Praxedes - **Coordenador Geral**
Representante Comunidade Científica

Alair Augusto Santos / Maria Lúcia Santos
Faculdade de Medicina - Depto Radiologia

Maria Nazareth C. Pinto / Alberto Esteves Gemal
Faculdade de Medicina - Depto Cirurgia

Renato Augusto M. Sá / Selma Maria A. Sias
Faculdade de Medicina - Depto Materno Infantil

Regina Helena S. Peralta / Andréa Alice da Silva
Faculdade de Medicina - Depto Patologia

Jano Alves de Souza / Pedro Ferreira M. Filho
Faculdade de Medicina - Depto Medicina Clínica

José Carlos Carraro Eduardo
Faculdade de Medicina – Repr. Colegiado

Carlos Dimas M.Ribeiro/Marcos Antônio A.Senna
Instituto de Saúde da Comunidade

Sérvio Túlio / Rogério Dultra
Faculdade de Direito

Ana Paula Black Veiga
Hospital Universitário Antônio Pedro

Rosangela Arrabal Thomaz
Faculdade de Medicina

José Plácido / Lígia Lobato
Representantes da Comunidade Usuária

Tereza C. A. Graça / Theresa C.L. Coutinho
Faculdade de Odontologia

Thelma B. Machado / Sabrina C. Elias
Faculdade de Farmácia

Denise Mafra / Daniele M. Ferreira
Faculdade de Nutrição

Valdecyr Herdy Alves / Luiz dos Santos
Faculdade de Enfermagem

Dilvani Oliveira Santos / Luiz G. Gawryszewski
Faculdade de Biologia

Tatiana Rangel Reis / Sulamita B. de Lima
Escola de Serviço Social

Luís Antônio C. Ribeiro / Gilvan Hansen
Instituto de Ciências Sociais – Depto Filosofia

Josemberg M. Andrade / Elton H. Matsushima
Instituto de Ciências Sociais – Depto Psicologia

Licínio E. Silva / Ana Beatriz M. Fonseca
Instituto de Matemática

CEP CMM/HUAP nº 057/2010

CAAE nº 0042.0.258.000-10

Do: Coordenador do CEP CMM/HUAP
A(o) Sr.(a) Pesquisador(a):

Assunto: Parecer sobre Projeto de Pesquisa

Sr.(a) Pesquisador(a)

Informo a V.Sª. que o Comitê de Ética em Pesquisa da Faculdade de Medicina / Hospital Universitário Antônio Pedro, constituído nos termos da Resolução nº 196/96 do Conselho Nacional de Saúde e devidamente registrado na Comissão Nacional de Ética em Pesquisa, recebeu, analisou e emitiu parecer sobre a documentação referente ao protocolo de pesquisa e seu respectivo Termo de Consentimento Livre e Esclarecido, conforme abaixo discriminado:

Título do Projeto:
"Avaliação clínica, microbiológica, imunológica e genética de crianças portadoras ou não de diabetes melito tipo I "

Pesquisadora Responsável:
Profª. Drª. Cristiane Duque

Pesquisadores Colaboradores:
Profª. Drª. Apoena de Aguiar Ribeiro , Profª. Drª. Rebeca de Souza Azevedo , Profª. Drª. Gabriela Alessandra da Cruz , Profª. Drª. Michelle Mikhael Ammari

Data: 11/06/2010

Parecer: *Aprovado*

Atenciosamente,

Prof. Herbert Praxedes
Coordenador

ANEXO 1 (B) - Documento de aprovação do adendo do projeto pelo Comitê de Ética em Pesquisa.

UNIVERSIDADE FEDERAL FLUMINENSE
Comitê de Ética em Pesquisa da Faculdade de Medicina / Hospital Universitário Antônio Pedro

Herbert Praxedes - **Coordenador Geral**
Representante Comunidade Científica

Alair Augusto Santos / Maria Lúcia Santos
Faculdade de Medicina - Depto Radiologia

Maria Nazareth C. Pinto / Alberto Esteves Gemal
Faculdade de Medicina - Depto Cirurgia

Renato Augusto M. Sá / Selma Maria A. Sias
Faculdade de Medicina - Depto Materno Infantil

Regina Helena S. Peralta / Andréa Alice da Silva
Faculdade de Medicina - Depto Patologia

Mauro Diniz Moreira / Sérgio Setúbal
Faculdade de Medicina - Depto Medicina Clínica

José Carlos Carraro Eduardo
Faculdade de Medicina – Repr. Colegiado

Carlos Dimas M.Ribeiro/Marcos Antônio A.Senna
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Hospital Universitário Antônio Pedro

Rosangela Arrabal Thomaz
Faculdade de Medicina

José Plácido / Lígia Lobato
Representantes da Comunidade Usuária

Tereza C. A. Graça / Theresa C.L. Coutinho
Faculdade de Odontologia

Thelma B. Machado / Sabrina C. Elias
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Instituto de Ciências Sociais – Depto Filosofia

Abrahão Santos / Elton H. Matsushima
Instituto de Ciências Sociais – Depto Psicologia

Licínio E. Silva / Ana Beatriz M. Fonseca
Instituto de Matemática

<u>CEP CMM/HUAP nº **057/2010**</u>

CAAE nº 0042.0.258.000-10

Do: Coordenador do CEP CMM/HUAP
A(o) Sr.(a) Pesquisador(a):

Assunto: Parecer sobre Projeto de Pesquisa
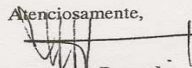
Sr.(a) Pesquisador(a)

Informo a V.Sª. que o Comitê de Ética em Pesquisa da Faculdade de Medicina / Hospital Universitário Antônio Pedro, constituído nos termos da Resolução nº 196/96 do Conselho Nacional de Saúde e devidamente registrado na Comissão Nacional de Ética em Pesquisa, recebeu, analisou e emitiu parecer sobre **ADENDO** ao protocolo de pesquisa e seu respectivo Termo de Consentimento Livre e Esclarecido, conforme abaixo discriminado:

Título do Projeto:
"Avaliação clínica, microbiológica, imunológica e genética de crianças portadoras ou não de diabetes melito tipo I "

Pesquisadora Responsável:
Profª. Drª. Cristiane Duque

Pesquisadores Colaboradores:
Profª. Drª. Apoena de Aguiar Ribeiro , Profª. Drª. Rebeca de Souza Azevedo , Profª. Drª. Gabriela Alessandra da Cruz , Profª. Drª. Michelle Mikhael Ammari, Aline Peçanha Muzy Dias.

Data: 06/05/2011

Parecer: *Aprovado o adendo em anexo*

Atenciosamente,

Prof. Herbert Praxedes
Coordenador

Renato A. Moreira de Sá
CRM 5251923-2

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ANEXO 2 – Termo de consentimento livre e esclarecido
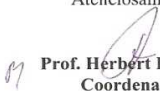
TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do Projeto: "AVALIAÇÃO CLÍNICA, MICROBIOLÓGICA, IMUNOLÓGICA E GENETICA DE CRIANÇAS PORTADORAS OU NÃO DE DIABETES MELITO TIPO I".

Pesquisadora Responsável: Profa. Dra. Cristiane Duque
Instituição a que pertence a Pesquisadora Responsável: Faculdade de Odontologia do Pólo Universitário de Nova Friburgo da Universidade Federal Fluminense
Telefones para contato: (22) 81178752

Nome do voluntário: _____

Idade: _____ anos   R.G. _____

Responsável legal: _____

R.G. Responsável legal: _____

O(A) Sr. (ª) está sendo convidado(a) a participar do projeto de pesquisa *"Avaliação clínica, microbiológica, imunológica e genética de crianças portadoras ou não de diabetes melito tipo I"*, de responsabilidade da pesquisadora Profa. Dra. Cristiane Duque.

Por favor, leia este termo cuidadosamente, pois, as informações a seguir irão descrever esta pesquisa e sua função nela como co-participante. Caso tenha qualquer dúvida sobre este estudo ou termo, você deverá esclarecê-la com a pesquisadora responsável pelo trabalho.

JUSTIFICATIVA E OBJETIVOS: A doença periodontal é uma das causas de perda precoce dos dentes e representa um problema de saúde pública de ampla distribuição mundial. Para entender as causas e os mecanismos de progressão dessa doença é necessário identificar precocemente as bactérias patogênicas e avaliar sua relação com a saúde gengival. Acredita-se que se um processo patológico acomete o periodonto (gengiva/osso) de uma criança, em longo prazo, esse pode ter influência sobre o periodonto do adulto. Assim, com o avanço da idade, a doença periodontal progride e pode comprometer severamente os tecidos que sustentam o dente. A presente pesquisa tem como objetivos avaliar a saúde gengival de crianças e verificar a presença de bactérias bucais relacionadas com a doença periodontal, além de alterações genéticas e no sistema de defesa do organismo.

Descrição do estudo: Para realização desta pesquisa serão selecionadas 80 crianças entre 7 a 13 anos de idade, apresentando ou não gengivite, diabéticas ou não. Todas as crianças serão avaliadas quanto à presença de cárie, biofilme (placa bacteriana) e gengivite e serão coletados o biofilme, fluido do sulco gengival e a saliva para avaliação laboratorial.

Riscos: este estudo não oferece nenhum risco aos indivíduos que participarem da pesquisa.

Benefícios Esperados: Ao ser voluntário, você além de estar contribuindo com a pesquisa, receberá maiores informações sobre promoção da saúde bucal, e seu filho, se tiver gengivite, receberá o tratamento e terá um controle mais eficaz com relação à presença de microrganismos. Todas as crianças, com ou sem gengivite, receberão instruções de higiene bucal e um kit com escova, pasta de dente e fio dental. Crianças com outros problemas bucais serão encaminhadas para triagem e tratamento na FOUFF/NF.

Forma de acompanhamento e assistência: Os voluntários têm garantia de que receberão respostas a qualquer pergunta ou esclarecimento sobre os procedimentos a serem realizados no seu filho, e aspectos pertinentes à pesquisa em qualquer momento. Se a criança apresentar alguma sensibilidade depois do tratamento, você poderá trazê-la à clínica para que os pesquisadores responsáveis tomem as providências necessárias. *Telefone para contato: Profa. Cristiane (22) 81178752

Forma de esclarecimento: Os voluntários têm garantia de que receberão informações, antes e durante a pesquisa, sobre a metodologia de estudo. E, se for de seu interesse receberão informações sobre os resultados finais obtidos.

Retirada do consentimento: Os voluntários têm liberdade de retirar o consentimento a qualquer momento e deixar de participar do estudo.

Garantia de sigilo: Os dados obtidos na pesquisa têm finalidade exclusivamente científica, sendo assegurada privacidade dos sujeitos da pesquisa. Os resultados deste projeto de pesquisa serão apresentados em

congressos e publicados em revistas científicas, porém a identidade do voluntário não será divulgada em nenhum momento.

Ressarcimento de despesas: Esta pesquisa será realizada na clínica de Odontopediatria e no laboratório de Ciências Básicas da Universidade Federal Fluminense, Faculdade de Odontologia do Pólo de Nova Friburgo. E o voluntário não correrá nenhum risco ou terá algum gasto para participar desta pesquisa.

Métodos alternativos para tratamento: não se aplica

Formas de minimização dos riscos associados: não se aplica. Somente se forem necessárias radiografias locais, a criança será protegida com avental e colar de chumbo.

Possibilidade de inclusão em grupo controle ou placebo: não se aplica

Formas de indenização (reparação a danos imediatos ou tardios) e o seu responsável: não se aplica

Eu, _____, RG nº _____, responsável legal por _____, RG nº _____ declaro ter sido informado e concordo com a sua participação, como voluntário, no projeto de pesquisa acima descrito.

Nova Friburgo, _____ de _____ de _____

_____   _____

Nome e assinatura do paciente ou seu responsável legal   Nome e assinatura do responsável por obter o consentimento

_____   _____

Testemunha   Testemunha

ANEXO 3 - Entrevista

NOME: _____

ENDEREÇO:_____

DATA DE NASCIMENTO: _____ **SEXO:** _____ **IDADE:**_____

RAÇA:_____**DIABÉTICO:** ( ) SIM ( ) NÃO **TELEFONE:**_____

NOME DO RESPONSÁVEL: _____**RG:** _____

HISTÓRIA MÉDICA GERAL
Sobre seu filho (a):

1- Seu filho (a) está com boa saúde?	Sim ( )	Não ( )
2- Quando ele (a) foi ao médico pela última vez?		
3- Ele (a) está em tratamento médico?	Sim ( )	Não ( )
4- Nome do médico: _____ Tel:_____		
End:_____ Cidade _____		
5- Ele (a) faz uso de algum medicamento?	Sim ( )	Não ( )
Qual (is) ? _____		
6- Ele (a) já foi submetido a alguma cirurgia?	Sim ( )	Não ( )
Qual (is) ? _____		
7- Ele (a) teve algumas das doenças abaixo:		
- Hepatite	Sim ( )	Não ( )
- Tuberculose	Sim ( )	Não ( )
- Artrite	Sim ( )	Não ( )
- Asma	Sim ( )	Não ( )
- Reumatismo	Sim ( )	Não ( )
- Febre reumática	Sim ( )	Não ( )
- Ele (a) tem problemas de pressão arterial?	Sim ( )	Não ( )
Qual? (Hipertensão arterial, Hipotensão arterial, Prolapso da válvula mitral, Infarte, Doenças congênitas, outras)		
- Ele (a) portador de próteses cardíacas?	Sim ( )	Não ( )
- Ele (a) teve alguma doença dermatológica?	Sim ( )	Não ( )
- Qual? (Lúpus eritematoso?), outra:_____		
- Sinusite	Sim ( )	Não ( )
- Anemia	Sim ( )	Não ( )
- Epilepsia (tontura, desmaios, convulsões)	Sim ( )	Não ( )
- Neoplasias	Sim ( )	Não ( )
- Problemas renais	Sim ( )	Não ( )
Outras: _____	Sim ( )	Não ( )
8- Ele (a) já teve hemorragia?	Sim ( )	Não ( )
- Ele (a) sangra muito quando se corta?	Sim ( )	Não ( )
- Ele (a) tem com freqüência manchas roxas no corpo?	Sim ( )	Não ( )
9- Ele (a) é alérgico ou tem reação adversa a:		
- Anestésico	Sim ( )	Não ( )
- Penicilina	Sim ( )	Não ( )
- Aspirina	Sim ( )	Não ( )
- Iodo	Sim ( )	Não ( )
- Barbitúricos, sedativos, tranqüilizantes, narcóticos	Sim ( )	Não ( )
- Outros_____	Sim ( )	Não ( )
10- Ela está grávida?	Sim ( )	Não ( )
- Toma anticoncepcionais?	Sim ( )	Não ( )
- Está amamentando?	Sim ( )	Não ( )
11- Ele (a) tem alguma doença, condição ou problema não listado acima que ache importante relatar?_____	Sim ( )	Não ( )

FATORES SISTÊMICOS DE RISCO ASSOCIADOS À DOENÇA PERIODONTAL

12- Ele (a) fuma?	Sim ( )	Não ( )
13 – Ele (a) tem diabetes?	Sim ( )	Não ( )

Tipo de diabetes. _____

Quanto tempo? _____

Esquema de insulina _____

No caso de resposta <u>negativa</u>:

- Urina mais que 6 vezes ao dia?	Sim ( )	Não ( )
- Sente muita sede?	Sim ( )	Não ( )
- Sente muita fome?	Sim ( )	Não ( )
- Tem problemas com cicatrização?	Sim ( )	Não ( )
14 – Ele (a) está estressado por algum motivo?	Sim ( )	Não ( )
- Insônia?	Sim ( )	Não ( )
- Aperta ou range os dentes?	Sim ( )	Não ( )
15 – Ele (a) tem alguma imunossupressão (neutropenia, drogas imunossupresoras)?	Sim ( )	Não ( )
16- Ele (a) tem AIDS?	Sim ( )	Não ( )
- Ele (a) já recebeu transfusão de sangue?	Sim ( )	Não ( )
- Ele (a) faz uso de drogas injetáveis?	Sim ( )	Não ( )

HISTÓRIA ODONTOLÓGICA/ PERIODONTAL

Ele (a) já reclamou que a gengiva sangra?	Sim ( )	Não ( )
Ele (a) já reclamou de dor na gengiva?	Sim ( )	Não ( )
Ele (a) tem mal hálito?	Sim ( )	Não ( )
Ele (a) já reclamou de gosto desagradável na boca?	Sim ( )	Não ( )
Ele (a) respira pela boca?	Sim ( )	Não ( )

<u>Hábitos de Higiene bucal:</u>

Quantas vezes ele (a) escova os dentes por dia? _____ Quem escova? _____

Qual o tipo de cerda da escova?

Qual dentifrício ele (a) está usando?

Ele (a) faz uso de bochechos? Qual? _____	Sim ( )	Não ( )
Ele (a) faz uso de fio/fita dental?	Sim ( )	Não ( )

Outros meios auxiliares de limpeza dental? Qual? _____

Ele (a) Usa de flúor? De que forma? ( ) bochechos na escola ( ) aplicação no dentista	Sim ( )	Não ( )

DECLARAÇÃO

Eu,_____R.G._____,responsá

vel pelo menor _____

declaro para todos os fins legais que as informações sobre o estado de saúde desta criança são verdadeiras e que nada omiti no questionário realizado.

Nova Friburgo, _____de _____ de 20___.

Assinatura: _____

Responsável

ANEXO 4 - Ficha clínica do projeto

Nome do paciente: _____**Sexo:** ( )M ( )F

Diabético: ( )sim ( )não **Data de nascimento:** ____/____/_____ **Data do exame:** ____/____/_____

ÍNDICE DE PLACA

	MAXILA						MANDÍBULA					
	16	55	11	21	65	26	36	75	31	41	85	46
V												
M												
D												
L												

ÍNDICE GENGIVAL

	MAXILA						MANDÍBULA					
	16	55	11	21	65	26	36	75	31	41	85	46
V												
M												
D												
L												

PROFUNDIDADE DE SONDAGEM

	MAXILA						MANDÍBULA					
	16	55	11	21	65	26	36	75	31	41	85	46
V												
M												
D												
L												

ceod/CPOD

	MAXILA												MANDÍBULA										
	55	54	53	52	51	61	62	63	64	65	SN	PC	75	74	73	72	71	81	82	83	84	85	
ceod																							
	MAXILA												MANDÍBULA										
	16	15	14	13	12	11	21	22	23	24	25	26	36	35	34	33	32	31	41	42	43	44	45	46
CPOD																								

ANEXO 5. Comprovante de envio do artigo para a revista Journal of Periodontology

JOURNAL OF
Periodontology

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Thank you for submitting your manuscript to *Journal of Periodontology*.

Manuscript ID:	JOP-13-0592
Title:	Relationship between microbiological, lipid and immunological profiles and the presence of gingivitis in type 1 diabetes mellitus children
Authors:	Dib, Mariana Duque, Cristiane Camargo, Gabriela Teixeira, Glaucia Machado, Thamiris Azevedo, Rebeca Mariano, Flávia Mattos-Graner, Renata
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