UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE ODONTOLOGIA

Julia Mourão Braga Diniz

ANÁLISE EPIDEMIOLÓGICA E DA RESPOSTA IMUNE PERIAPICAL EM PACIENTES PRÉ E PÓS TRANSPLANTE DE CÉLULAS TRONCO HEMATOPOIÉTICAS E DE FÍGADO PORTADORES DE INFECÇÕES ENDODÔNTICAS

BELO HORIZONTE
2017

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Tese apresentada ao Colegiado do Programa de Pós-Graduação da Faculdade de Odontologia da Universidade Federal de Minas Gerais, como requisito parcial para obtenção do grau de Doutor em Odontologia – área de concentração em Endodontia

Orientador: Prof. Dr. Antônio Paulino Ribeiro Sobrinho
Co-orientadora: Profa. Dra. Leda Quercia Vieira

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UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

FOLHA DE APROVAÇÃO

ANÁLISE EPIDEMIOLÓGICA E DA RESPOSTA IMUNE PERIAPICAL EM PACIENTES PRÉ E PÓS TRANSPLANTE DE CÉLULAS TRONCO HEMATOPOIÉTICA E DE FÍGADO PORTADORES DE INFECÇÕES ENDODÔNTICAS

JULIA MOURÃO BRAGA DINIZ

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

Aprovada em 03 de agosto de 2017, pela banca constituída pelos membros:

Prof(a). Antônio Paulino Ribeiro Sobrinho - Orientador
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Belo Horizonte, 3 de agosto de 2017.

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA

ATA DA DEFESA DE TESE DA ALUNA
JULIA MOURÃO BRAGA DINIZ

Aos 03 dias de agosto de 2017, às 14:00 horas, na sala 3403 da Faculdade de Odontologia da Universidade Federal de Minas Gerais, reuniu-se a Comissão Examinadora composta pelos professores Antonio Paulino Ribeiro Sobrinho (Orientador) – FO/UFMG, Fernando de Oliveira Costa – FO/UFMG, Leandro Napier de Souza – FO/UFMG, Taia Maria Berto Rezende – Universidade Católica de Brasília e Brenda Paula Figueiredo de Almeida Gomes – UNICAMP para julgamento da tese de Doutorado em Odontologia, área de concentração em Endodontia, intitulada: **Análise epidemiológica e da resposta imune periapical em pacientes pré e pós transplante de células tronco hematopoiética e de fígado portadores de infecções endodônticas**. O Presidente da Banca, abriu os trabalhos e apresentou a Comissão Examinadora. Após a exposição oral do trabalho pela aluna e arguição pelos membros da banca, a Comissão Examinadora considerou a tese:

(X) Aprovada

( ) Reprovada

Finalizados os trabalhos, lavrou-se a presente ata que, lida e aprovada, vai assinada por mim e pelos demais membros da Comissão. Belo Horizonte, 03 de agosto de 2017.

Prof(a). Antonio Paulino Ribeiro Sobrinho

Prof(a). Fernando de Oliveira Costa

Prof(a). Leandro Napier de Souza

Prof(a). Taia Maria Berto Rezende

Prof(a). Brenda Paula Figueiredo de Almeida Gomes

DEDICATÓRIA

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AGRADECIMENTOS

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RESUMO

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As patologias pulpares e perirradiculares abrangem muitas áreas do conhecimento, tais como a epidemiologia, microbiologia e imunologia. Por sua vez, os estudos que correlacionam a necessidade de intervenção endodôntica com as alterações sistêmicas são escassos na literatura. O Transplante de Células Tronco Hematopoiéticas (TCTH) envolve a ablação das células anormais ou malignas com altas doses de quimioterapia, com ou sem radioterapia corpórea total, o que leva a um estado de imunossupressão. Doenças hepáticas são muito comuns e as principais causas relacionadas são: infecções, uso abusivo de álcool e distúrbios metabólicos. Essas alterações sistêmicas tornam o indivíduo mais susceptível às infecções, que podem, inclusive, culminar com o seu óbito. O objetivo do presente estudo é avaliar as necessidades de tratamento endodôntico e caracterizar a resposta imune periapical desses indivíduos. Neste estudo, 188 indivíduos na fase pré e pós Transplante de Células Tronco Hematopoiéticas (TCTH) e 120 indivíduos na fase pré transplante de fígado (TF) foram selecionados. Os dados pessoais e sistêmicos destes pacientes foram correlacionados com a necessidade de tratamento endodôntico. Aqueles indivíduos que apresentavam necessidade de tratamento endodôntico tiveram seus canais radiculares analisados quanto ao perfil imunológico. Pacientes sem comprometimento sistêmico foram também avaliados para que se confrontassem os dados. Os espécimes clínicos foram coletados em dois momentos distintos, imediatamente após os procedimentos de limpeza e formatação e 7 dias após. Utilizando-se o Real Time PCR, avaliou-se a expressão das citocinas e quimiocinas TNF-α, IL-1β, IL- 10, IFN-γ, IL-6, CCL2, CCL4, CXCR4 e dos fatores angiogênicos VEGF e Angiopoetina. No grupo de TCTH, pode-se observar que a maioria dos indivíduos na fase pré-transplante de medula óssea apresentaram valores de plaquetas e hemoglobina abaixo dos valores de referência no primeiro atendimento. Por sua vez, a maioria dos pacientes pré transplante de fígado apresentaram os valores de plaquetas e hemácias abaixo dos valores de referência no momento do primeiro atendimento. A maioria dos pacientes faz uso de algum tipo de medicamento, 75,5% e 92,5%, no grupo TCTH e TF, respectivamente. Os

antibióticos são utilizados por 32% e 51,8% dos pacientes pré e pós TCTH enquanto os diuréticos representam o medicamento mais utilizado pelos pacientes pré TF (90,8%). Foi constatada a necessidade de tratamento endodôntico, nos pacientes pré e pós TCTH, de 24,3% e 24,7% respectivamente. A necessidade de tratamento endodôntico observada nos pacientes pré TF foi de 20,7%. Ao comparar o grupo de estudo de pacientes pré TCTH com o grupo controle, os resultados em relação à expressão de citocinas e quimiocinas foram: Um aumento significativo na expressão das citocinas pró-inflamatórias TNF-α e IFN-γ foi observado após a instrumentação do SCR (dia 7), quando comparado ao dia 0, em ambos os grupos; um aumento na expressão de IL-1β e de IL-10, após a instrumentação do SCR, também foi observada no grupo pré TCTH; houve um aumento na expressão do receptor CXCR-4, no dia 7, no grupo controle; e a quimiocina MCP-1 não foi detectada no grupo pré TCTH. Ao comparar o grupo de estudo de pacientes pré TF com o grupo controle, os resultados em relação à expressão de citocinas, quimiocinas e fatores angiogênicos foram: Um aumento significativo na expressão das citocinas pró inflamatórias TNF-α e IFN-γ foi observado após a instrumentação do SCR (dia 7), quando comparado ao dia 0 no grupo controle; uma maior expressão de TNF-α no dia 7 também foi observada no grupo de TF; a expressão de IL-1β foi maior no grupo pré TF no dia 0; um aumento significativo de AGT, após a instrumentação do SCR, foi observado no grupo controle. Pode-se concluir que: a) A porcentagem de indivíduos com necessidade de tratamento endodôntico nos grupos de estudo é elevada; b) A maioria dos pacientes faz uso de algum tipo de medicamento. Os antibióticos representam a medicação mais utilizada no grupo TCTH enquanto os diuréticos representam a medicação mais utilizada no grupo TF; c) Indivíduos pré TCTH e pré TF, em sua maioria apresentam baixa contagem de células o que pode estar relacionado com o comprometimento imune desses pacientes; d) A expressão gênica das citocinas e quimiocinas demonstra nos pacientes pré TCTH uma resposta pró-inflamatória e anti-inflamatória eficaz, similar a observada no grupo controle e e) A expressão gênica das citocinas, quimiocinas e dos fatores angiogênicos sugere que o comprometimento

hepático não interfere na resposta imune periapical uma vez que uma resposta similar foi observada no grupo controle.

Palavras Chave: Tratamento endodôntico, transplante de células tronco hematopoiéticas, transplante de fígado, microbiologia, imunologia e epidemiologia.

ABSTRACT

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Pulpo-periapical pathologies cover many areas of knowledge, including epidemiology, microbiology and immunology. Literature lacks of studies about main disease that lead to hematopoietic stem cell transplant interference and their treatment. Hematopoietic Stem Cell Transplant (HSCT) involves high dose chemotherapy with or without full body radiotherapy ablation of abnormal or malignant cells, leading patient to a immunosuppression state. Liver diseases are a very common health matter, and the main underlying causes include infections, alcohol abuse and lipid and carbohydrate metabolic disorders. Those conditions make the subject more susceptible to infections, provoking considerable morbidity or even death. In this work, 188 pre and post transplant (TCTH) individuals and 120 pre liver transplant subjects were selected. Patients' personal and systemic data were correlated with endodontic treatment need. Subjects that needed endodontic treatment had the root canals of those teeth analyzed for their immunological profile. Healty individuals (that needed endodontic therapy) were selected as controls for comparison. Samples were collected at two times points, immediately after cleaning and shaping the root canal system and 7 days later. Using Real Time PCR, we evaluated the cytokines and chemokines expression of TNF-α, IL-1β, IL- 10, IFN-γ, IL-6, CCL2, CCL4, CXCR4, VEGF angiogenic factors and angiopoietin. In the TCTH group, most individuals showed platelets and hemoglobin values bellow reference values before transplant. Furthermore, most HSCT pre transplant patients exhibited platelet and red blood cells counts below the reference values at first appointment time. Most of the analyzed patients used some medication, being 75.5% and 92.5%, at TCTH and TF groups, respectively. Antibiotics were taken by 32% and 51.8% of pre and post TCTH pacients whilst diuretics represent most taken medication by pre TF patients (90.8%). It was attested endondontic treatment need, in pre e post TCTH patients, of 24.3% e 24.7% respectively. Endondontic treatment need observed in pre TF patients was 20.7%. Comparing pre TCTH patients com with control, the results regarding chetokines and chemokines expression were: significantly increased expression levels of TNF-α and IFN-γ on day 7; the mRNA levels of IL-1-β and IL-10 in the

bone marrow group increased in the samples from day 7; chemokine receptor CXCR4 levels increased in samples obtained from the day 7 control group; the chemokine CCL-2/MCP-1 was not detected in patients undergoing HSCT. Comparing pre TF patients with control, regarding cytokines, chemokines and angiogenic factors expression were: in control/ health group, a significantly increased expression of proinflammatory cytokines IFN-γ and TNF-α was observed in teeth with restrained bacterial loads (day 7); larger expression of TNF-α at day 7 were also observed in the TF group; significant increase in mRNA levels of AGT in teeth with restrained bacterial load (day 7) compared to the first collection in control individuals. It can be concluded that: a) The percentage of individuals with endodontic treatment need is high at all astudied groups; b) Most of the analysed patients used some medication being antibiotics most used drug in TCTH group whilst diuretics represent the most used medicine in TF group; c) Pre TCTH and pre TF individuals, mostly present low blood cell count what can be related to these patients immune depression; d) Genic chemokine and cytokine expression showed that pre TCTH patients have a pro and anti-inflammatory efficient response, similar to control and e) Genic chemokine, cytokine, and angiogenic expression suggest that liver impairment did not compromise periapical immune response once a similar response was observed in control.

Key words: Endodontic treatment, Hematopoietic Stem Cell Transplant, Liver transplant, microbiology, immunology and epidemiology.

LISTAS (TABELAS, ABREVIATURAS E SIGLAS)

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SIGLAS

ANG: *Angiopoetina*
CD4: *cluster of differenciation 4*
CD8: *cluster of differenciation 8*
CCL: *Chemokine (C-C motif) ligand*
CCR: *chemokine (C-C motif) receptor*
CXCR: *C-X-C chemokine receptor type*
DECH - *Doença do enxerto contra o hospedeiro*
DNA: *Deoxyribonucleic acid*
EDTA - *Acído etilenodiamino tetra-acético*
GAPDH: *Glycer-aldehyde 3-phosphate dehydrogenase*
HC: *Hospital das Clínicas*
IFN-: *Interferon*
IL-: *Interleukin*
MCP-1: *monocyte chemotactic protein-1*
mRNA: *Messenger RNA*
PAOPT: *Programa de Assistência Odontológica a Pacientes Transplantados*
PCR: *polymerase chain reaction*
RNA: *Ribonucleic acid*
SCR: Sistema de canais radiculares
SD: Standart deviation
TCD4+: *Tcell CD4*
TCD8+: *Tcell CD8*
TCLE: *Termo de consentimento livre e esclarecido*
TCTH - *Transplante de Células Tronco Hematoipoéticas*
TGF: *Transforming growth factor*
TF: Transplante de fígado
Th: T helper cell
TNF: *Tumor necrosis factors*
Treg: *Regulatory T cells*
UFMG: *Universidade Federal de Minas Gerais*
VEGF: *fator de crescimento vascular endotelial*

SUMÁRIO

INTRODUÇÃO

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As alterações pulpares e perirradiculares são normalmente resultado do envolvimento direto ou indireto de microrganismos da cavidade oral, que são essenciais à progressão e a perpetuação das diferentes formas de alterações periapicais (1-3). Havendo uma infecção microbiana instalada no SCR, o sistema de defesa do hospedeiro procura localizá-la nos arredores do ápice radicular (4). A resposta inflamatória, que aí se processa, recruta células imunocompetentes para conter e impedir a disseminação dessa infecção para outros sítios, culminando com a formação de uma lesão crônica e a concomitante reabsorção dos tecidos de suporte periodontal adjacentes (5). Nas últimas décadas, houve fortes evidências de que muitos dos efeitos patogênicos microbianos sobre os tecidos periapicais operam-se de forma indireta, via estimulação de mediadores solúveis derivados do hospedeiro, como as citocinas e quimiocinas (6). Daí o grande interesse em se conhecer esses mediadores e seus efeitos sobre as células imunocompetentes (7).

Nas lesões perirradiculares humanas, encontramos uma grande variedade de células, dentre elas: os linfócitos TCD4+ e TCD8+, macrófagos, células plasmáticas, mastócitos, eosinófilos. As células T, entretanto, são as mais numerosas nessas lesões (8). Os linfócitos TCD4+ e CD8+, após o contato com antígenos ou de serem estimuladados por outras células inflamatórias, podem produzir uma grande variedade de citocinas (9). As células TCD4+ atualmente são subdivididas em vários subgrupos que incluem as células: Th1, Th2, Th17 e T regulatórias (Treg) (10).

A resposta Th1 caracteriza-se pela produção de IFN-γ, IL-12, IL-2, e TNF, envolvendo-se na progressão das lesões e destruição óssea perirradicular (6, 8). A resposta celular do tipo 2 (Th2) produz IL-4, IL-5, IL-6, IL-10 e IL-13 que estão relacionadas aos processos de cura e reparo da área afetada (11, 12). Esses dois subgrupos, Th1 e Th2, apresentam regulação cruzada, através de liberação de citocinas antagônicas como a IL-10 e o fator de crescimento tumoral (TGF-β). Ou seja, as células Th1 inibem a resposta Th2 pela produção de IFN-γ e IL-12, enquanto as células Th2 inibem a geração de uma resposta Th1 produzindo citocinas como a IL-4 e IL-10 (12).

O subgrupo Th17 produz a IL-17, citocina pró-inflamatória com atuação em várias células da resposta inata, e é considerada ponte entre a resposta adaptativa e inata (13). Entretanto, as células Treg, produtoras de TGF-β e IL-10 possuem um efeito inibitório sobre a reabsorção óssea durante a formação e diferenciação dos osteoclastos, além de atuarem na regulação da resposta imune contra a infecção (8).

As quimiocinas possuem um papel de destaque no processo inflamatório uma vez que funcionam como agentes quimiotáticos e promovem uma resposta imune inicial para diferentes tipos de patógenos, através do recrutamento de células efetoras apropriadas para o sítio inflamatório, incluindo o recrutamento de diferentes células Th1 e Th2 (14). CCL2/MCP-1 tem sido associado ao recrutamento de células inflamatórias e tem ação sobre a diferenciação de células T efetoras, uma vez que leva a diminuição da produção da IL-12 pelos macrófagos e a supressão da resposta Th1(15).

A resposta inflamatória não pode ocorrer sem os componentes vasculares. A angiogênese tem sido apontada como fator essencial na patogênese das lesões periapicais crônicas, estando relacionada ao estabelecimento e manutenção da lesão (16). Os vasos sangüíneos neoformados suprem a contínua demanda de nutrientes e oxigênio pelas células em proliferação e contribuem para a inflamação por serem uma fonte constante de citocinas, quimiocinas e proteases e por estar relacionada ao reparo tecidual (16). O fator de crescimento vascular endotelial (VEGF) é considerado um regulador chave da permeabilidade vascular e um dos principais indutores da angiogênese (17, 18). Ele é o fator essencial para a diferenciação do sistema vascular e um potente mitógeno para as células endoteliais, promovendo a sua proliferação e migração (16, 19). Alem disso, a expressão de VEGFs e seus receptores em osteoblastos e osteoclastos sugere a ligação entre o crescimento vascular e remodelação óssea (19). Outro notável fator envolvido na angiogênese é a Angiopoetina que se liga aos receptores das células endoteliais, fazendo com que elas produzam fatores de migração, proliferação e diferenciação para as células periendoteliais que irão envolver os tubos de endotélio, sustentar e definir a arquitetura dos vasos, bem como permitir a maturação do vaso (20).

O Transplante de células tronco hematopoiéticas (TCTH) é amplamente utilizado no tratamento de doenças hematológicas malignas, leucemia aguda e crônica, anemia aplásica, síndromes mielodisplásicas, imunodeficiência combinada severa, linfomas e alguns tumores sólidos como o câncer de mama. O TCTH envolve a eliminação das células malignas com uma alta dose de quimioterapia, com ou sem a irradiação corpórea total, seguida pela infusão de células normais mieloproliferativas (21-24). Mais de 80% pacientes transplantados desenvolvem pelo ao menos uma infecção no pós TCTH e 40% das mortes são devido a complicações de infecções que ocorrem isoladamente ou após a rejeição do enxerto (25). A prevenção ou redução do risco de complicações sistêmicas em pacientes que recebem o transplante requer a estabilização ou eliminação de infecções bucais antes do início do transplante ou da terapia mielossupressora, pois a condição de imunossupressão do paciente favorece a agudização de problemas bucais prévios, no transcorrer do TCTH (26). Os fatores de risco associados à ocorrência de infecções estão relacionados a doença maligna, a presença de infecções crónicas ou latentes, ao tipo de transplante, a fonte das células estaminais, a utilização de agentes antimicrobianos, a perda da barreira mucosa, a imunossupressão e a mielossupressão induzidas após o TCTH e a doença do enxerto contra o hospedeiro (DECH) (27). Dois mecanismos desempenham um papel importante no risco de infecção. Um depende de defesas não específicas, tais como a integridade das barreiras de superfície e a presença de agentes antimicrobianos sistêmicos ou salivares. A outra grande defesa contra infecções é o sistema imune que, após o transplante, apresenta praticamente todos os componentes deficientes ou suprimidos pela terapia imunossupressora para impedir a DECH. A atividade de granulócitos, monócitos, macrófagos, células natural killer, e das células T tornam-se deficientes após o transplante (28). Os pacientes submetidos ao TCTH são normalmente pancitopênicos antes e imediatamente depois do transplante e neutropênicos por cerca de 6 a 12 meses depois do enxerto. Durante esta fase neutropênica, a infecção bucal pode causar graves conseqüências, podendo comprometer o sucesso do transplante (29). As infecções no período pós-

transplante tardio são atribuídas à imunodeficiência persistente, geralmente causada pela DECH crônica ou por seu tratamento.

Doenças hepáticas são muito comuns e as principais causas relacionadas são: infecções, uso abusivo de álcool e distúrbios metabólicos. A insuficiência hepática ocorre quando, rapidamente ou gradualmente no decorrer de anos, o fígado perde a sua capacidade de funcionamento. Nesses casos, o transplante hepático é a única solução (30). A maioria, entre 60% a 80%, dos pacientes submetidos ao transplante de fígado desenvolve algum tipo de infecção o que pode comprometer o sucesso do transplante. As infecções ainda representam a maior causa de morte entre os pacientes submetidos ao transplante de fígado (31). Estudos demonstraram que pacientes com doenças hepáticas crônicas, particularmente pacientes com cirrose hepática e hepatite C, apresentam uma higiene oral deficiente e uma maior incidência de carie e doença periodontal (32-36). Além disso, esses pacientes são mais suscetíveis a infecções bacterianas o que pode levar à bacteremia e eventualmente à morte (37). Essa maior suscetibilidade à infecções pode estar relacionada ao comprometimento sistêmico desses pacientes (35).

A cárie dentária e a doença periapical são as duas condições bucais mais comuns que podem acarretar sérias complicações sistêmicas (38). As lesões sintomáticas periapicais de natureza endodôntica constituem um potencial sítio de infecção. Adicionalmente, infecções dentárias foram associadas a varias condições e doenças sistêmicas, tais como: diabetes, doenças cardiovasculares e respiratórias e ao parto prematuro (39-41). Dentes infectados representam uma fonte de agressão microbiana persistente (42). Para evitar tais ocorrências e para reduzir a potencial morbidade causada por infecções dentárias em pacientes imunocomprometidos, a maioria dos centros de transplante recomenda um exame oral como parte do processo de avaliação com o objetivo de detectar e eliminar possíveis focos de infecção oral para reduzir a bacteremia e a eventual morbidade (43).

Sabe-se que pacientes com doenças hepáticas crônicas e os pacientes com doenças que levam ao transplante de medula, devido ao estado de imunossupressão, possuem um alto risco de desenvolverem infecções oportunistas incluindo as lesões orais. Compreender, pois, a necessidade de

tratamento endodôntico desses pacientes e, além disso, caracterizar a resposta imune perirradicular nesses pacientes contribuirá para a manutenção da saúde dos mesmos.

OBJETIVOS

2.1 Objetivo geral

- Avaliar a necessidade de tratamento endodôntico em pacientes pré e pós TCTH e, também, dos pacientes pré transplante de fígado, atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG, correlacionando-a aos seus dados demográficos e sistêmicos;
- Caracterizar a expressão gênica de citocinas e quimiocinas nos tecidos perirradiculares de pacientes pré TCTH e dos pacientes pré transplante de fígado, atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG.

2.2 Objetivos especificos:

- Identificar o perfil epidemiológico dos pacientes pré e pós TCTH atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG;
- Identificar e comparar a necessidade de tratamento endodôntico dos indivíduos na fase pré e pós TCTH atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG;
- Comparar os valores de neutrófilos, plaquetas e hemoglobina no momento do atendimento apresentados pelos indivíduos pré e pós TCTH atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG;
- Identificar o perfil epidemiológico dos pacientes com doenças hepáticas, na fase pré transplante de fígado, atendidos no Programa de Assistência Odontológica a Pacientes Transplantados da UFMG;
- Identificar a necessidade de tratamento endodôntico dos indivíduos com doenças hepáticas, na fase pré transplante de fígado, atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG;
- Apresentar os valores de neutrófilos, eosinófilos, monócitos, basófilos, plaquetas e hemoglobina no momento do primeiro atendimento apresentados pelos indivíduos com doenças

hepáticas, na fase pré transplante de fígado, atendidos no Programa de assistência odontológica a pacientes transplantados da UFMG;

- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10 e das quimiocinas CCL-2/MCP-1, CCL-4 e CXCL-4 nas lesões perirradiculares dos indivíduos na fase pré TCTH, logo após a instrumentação do SCR;
- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10 e das quimiocinas CCL-2/MCP-1, CCL-4 e CXCL-4 nas lesões perirradiculares dos indivíduos na fase pré TCTH, uma semana após a instrumentação do SCR;
- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10, IL-6, das quimiocinas CCL-2/MCP-1 e dos fatores angiogênicos VEGF e ANG nas lesões perirradiculares dos indivíduos na fase pré transplante de fígado, logo após a instrumentação do SCR;
- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10, IL-6, das quimiocinas CCL-2/MCP-1 e dos fatores angiogênicos VEGF e ANG nas lesões perirradiculares dos indivíduos na fase pré transplante de fígado, uma semana após a instrumentação do SCR;
- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10, IL-6, das quimiocinas CCL-2/MCP-1, CCL-4 e CXCL-4 e dos fatores angiogênicos VEGF e ANG nas lesões perirradiculares de indivíduos sem comprometimento sistêmico, logo após a instrumentação do SCR;
- Caracterizar, por PCR em tempo real, a expressão das citocinas IL-1β, IFN-γ, TNF-α, IL-10, IL-6, das quimiocinas CCL-2/MCP-1, CCL-4 e CXCL-4 e dos fatores angiogênicos VEGF e ANG nas lesões perirradiculares de indivíduos sem comprometimento sistêmico, uma semana após a instrumentação do SCR.

METODOLOGIA EXPANDIDA

3- Metodologia expandida

3.1- Fase I- Análise Epidemiológica

3.1.1 Pacientes

A população estudada foi composta por pacientes atendidos no Programa de Assistência Odontológica a Pacientes Transplantados da UFMG (PAOPT-UFMG) entre março de 2011 a março de 2016. Foram excluídos do estudo os pacientes cujos prontuários não puderam ser obtidos. Os pacientes foram divididos em dois grupos:

A) pacientes com doenças que levam ao TCTH; e

B) pacientes com doenças que levam ao transplante de fígado.

No grupo A, cento e oitenta e oito indivíduos foram incluídos na análise sendo 85 pacientes transplantados e os outros 103 pacientes com necessidade de TCTH. No grupo B, cento e vinte indivíduos foram incluídos na análise, todos na fase pré-transplante.

Os pacientes pré TCTH receberam o regime condicionante para o TCTH baseado no protocolo específico da Unidade de Transplante de Células-Tronco Hematopoiética do HC-UFMG. Os pacientes pós TCTH receberam o tratamento odontológico de manutenção da saúde bucal.

Os pacientes pré-transplante de fígado receberam o regime condicionante baseado no protocolo proposto pelo PAOPT-UFMG.

3.1.2 Dados pessoais e características clínicas

As informações referentes aos dados pessoais e as características clínicas dos pacientes foram obtidas a partir dos prontuários médicos. As características pessoais e as variáveis clínicas avaliadas foram: idade, sexo, fase do transplante, doença primária, tipo de transplante, necessidade de tratamento endodôntico, número de tratamentos endodônticos realizados, medicamentos em uso, dados do hemograma no momento do atendimento. Informações omitidas ou incompletas foram reportadas nesse estudo como “Dados perdidos/omitidos”.

3.1.3 Análise estatística dos dados

Foi realizada análise descritiva, utilizando a média e o desvio-padrão para as variáveis quantitativas. O teste estatístico Kolmogorov-Smirnov foi utilizado para testar a normalidade e o teste Chi-square foi utilizado para verificar se havia associação estatística entre as variáveis.

3.2 Fase II - Análise Imunológica

Os pacientes que apresentaram necessidade de tratamento endodôntico receberam atendimento clínico e, nesse momento, coletas dos espécimes foram realizadas para se analisar o perfil imunológico dessas infecções.

3.2.1 Grupos amostrais

O estudo foi composto pelos seguintes grupos:

- ✓ Grupo Experimental 1 - pacientes portadores de dentes com necrose pulpar com necessidade de TCTH.
- ✓ Grupo Experimental 2 - pacientes portadores de dentes com necrose pulpar com necessidade de transplante de fígado.
- ✓ Grupo Controle - pacientes portadores de dentes com necrose pulpar sem nenhum comprometimento sistêmico.

3.2.2 Seleção dos pacientes

Foram selecionados pacientes que apresentaram dentes com necrose pulpar. O diagnóstico de necrose pulpar foi realizado através de exames clínicos e radiográficos. Para o grupo experimental 1, dez pacientes foram selecionados e, para o grupo experimental 2, onze pacientes foram selecionados. O grupo controle foi composto por 11 pacientes. A cada paciente foi apresentado um termo de consentimento livre esclarecido (TCLE), constando as informações sobre o objetivo da presente pesquisa. As coletas foram realizadas naqueles indivíduos que concordaram em participar do estudo e que possuíam um elemento dental com indicação de tratamento endodôntico que se enquadrasse nos grupos amostrais propostos acima.

3.2.3 Coleta dos espécimes clínicos para identificação das citocinas.

O dente selecionado, após os procedimentos clínicos iniciais, teve sua coroa clínica completamente isolada (isolamento absoluto). A coroa foi desinfectada de acordo com o método proposto por Möller (1966) (água oxigenada 10 volumes por 5 minutos, tintura de iodo a 5% por 5 minutos e, posteriormente, tiossulfato de sódio a 5%). O tratamento foi realizado utilizando as limas ProTaper Universal (Dentsply Maillefer, Ballaigues, Switzerland) e irrigação com hipoclorito de sódio na concentração de 2.5%. As amostras foram coletadas imediatamente após a instrumentação. Para isso, três cones de papel absorvente foram inseridos, um de cada vez, no interior do conduto de maior calibre, ultrapassando o forame radicular em um milímetro e mantido por 2 minutos, para que o mesmo entre em contato com os tecidos perirradiculares. Posteriormente, 4mm da porção apical de cada cone foi seccionada, os cones foram inseridos em um eppendorf e armazenados no freezer - 80°. Nenhuma medicação intracanal foi utilizada e o selamento da cavidade de acesso foi realizado com cotosol. Após 7 dias, o selamento foi removido e, novamente, três cones de papel absorvente foram inseridos no conduto de maior calibre, seccionados a 4mm, inseridos no eppendorf e armazenados no freezer -80°.
Posteriormente, os canais foram obturados utilizando a técnica da compactação lateral. Nenhuma sintomatologia foi reportada pelos pacientes no momento da obturação.

3.2.4 Etapa laboratorial - Identificação da expressão de citocinas

3.2.4.1 Extração do RNA

A cada eppendorf foi adicionado 500 µl de TRIZOL (GIBCO BRL Laboratories, Grand Island, N.Y., EUA) e, utilizando um triturador, foi realizada a homogeinização da amostra. As amostras foram incubadas por 10 minutos no gelo, para permitir a completa dissociação de complexos nucleoprotéicos. Posteriormente, 100 µl de Clorofórmio de alta qualidade foram adicionados em cada amostra. As amostras foram novamente incubadas por 3 minutos no gelo e centrifugadas a 12.000xg por 15min a 2° a 8°C. Após a centrifugação a mistura foi separada em uma fase inferior rosa (fenolclorofórmio), uma fase intermediária e uma fase aquosa transparente. A fase aquosa foi transferida

para microtubos contendo 250µl de Isopropanol (MERK) de altíssima qualidade, vortexadas e incubada no gelo. Após 10 minutos, as amostras foram centrifugadas por 12.000 xg por 10 minutos a 2°C a 8°C. Nesse momento, foi observada a precipitação de um pellet transparente no lado e no fundo do tubo e o sobrenadante foi descartado. Posteriormente, foi adicionando 500 µl de etanol 75% a cada amostra, os microtubos foram levados ao vortex para soltar o pellet e novamente as amostras foram centrifugadas a 7.500 xg por 5 minutos de 2° a 8°C. Os sobrenadantes foram novamente descartados e os microtubos foram emborcados sobre um papel de filtro, por 10 minutos para secar o pellet. Após os 10 minutos, os pellets foram ressuspendidos em 50 µl de água de alta qualidade (DEPC) e armazenadas em freezer a -70°C.

3.2.4.2 Quantificação do mRNA

A quantificação do mRNA foi realizada no Nanodrop, adicionando 2 µl de amostra no local adequado do equipamento.

3.2.4.3 Preparo de cDNA por Transcrição Reversa

Foi preparada uma solução com os seguintes reagentes:

1. DNTPs (mistura a 2,5 mM).....1,25 µL
2. Reverse Transcriptase Buffer..... 2,50 µL.
3. 0,1 M Dithiothreitol (DTT).....1,00 µL
4. H_2O..... 0,25 µL
5. Oligo dT_{15} 1/10 - 7.5 ρMoles.....1,00 µL

As amostras de RNA foram acrescidas de 12 µL da solução acima descrita, aquecidas a 70ºC por 5 minutos e subsequentemente resfriadas em gelo, por 5 minutos. As amostras foram acrescidas de 3 µL de Transcriptase Reversa (12,5 U/reação – 25 U/reação), e deixadas, em temperatura ambiente, por 5 minutos. As amostras foram incubadas a 37°C durante uma hora, aquecidas a 90ºC por 5 minutos e resfriadas em gelo por 5 minutos, sendo a seguir estocadas a -20ºC.

3.2.4.4 Detecção e quantificação das citocinas

A quantificação das citocinas IL-1β, IL-10, IL-6, IFN-γ, TNF-α, das quimiocinas CCL2/MCP-1, CCL-4 e do CXCR-4 e dos fatores angiogênicos VEGF e ANG foram realizada pelo Real Time PCR, amplificando-se o mRNA e quantificando-se o cDNA provenientes das amostras clínicas. No grupo experimental 1 foram quantificadas as citocinas IL-1β, IFN-γ, TNF-α, IL-10 e as quimiocinas CCL-2/MCP-1, CCL-4 e CXCL-4. No grupo experimental 2, as citocinas quantificadas foram IL-1β, IFN-γ, TNF-α, IL-10, IL-6 e as quimiocinas CCL-2/MCP-1 e os fatores angiogênicos VEGF e ANG. No grupo controle, todas as citocinas, quimiocinas e fatores angiogênicos citados acima foram quantificados.

Tabela 1: Sequência dos *Primers*

Gene	Sense and antisense	Mt (°C)	bp
GAPDH	5'-GCA CCA CCA ACT GCT TAG CA-3'	80	96
	5'-TGG CAG TGA TGG CAT GGA GGA-3'		
IFN- γ	5'-GAA CTG TCG CCA GCA GCT AAA-3'	80	95
	5'-TGC AGG CAG GAC AAC CAT TA-3'		
IL-1β	5'-TGG CAG AAA GGG AAC AGA A- 3'	73	59
	5'-ACA ACA GGA AAG TCC AGG CTA- 3'		
TNF-α	5'-TTC TGG CTC AAA AAG AGA ATT G- 3'	76	73
	5'-TGG TGG TCT TGT TGC TTA AGG- 3'		
IL-10	5'-GGT TGC CAA GCC TTG TCT GA- 3'	81	107
	5'-TCC CCC AGG GAG TTC ACAT- 3'		
CCL2/MCP-1	5'-AAG ACC ATT GTG GCC AAG GA- 3'	80	93
	5'-CGG AGT TTG GGT TTG CTT GT- 3'		
IL-6	5'- GGA GAC TTG CCT GGT GAA- 3'	80	76
	5'- CTG GCT TGT TCC TCA CTA CTC-3'		
AGT	5'ACAGTTTGGCAATTGGAAGCA3'	65	152
	5' CACCCAGATGACTCCAAGATCAG3'		
VEGF	5′-ATC TGC ATG GTG ATG TTG GA-3′	71	214
CCL4	5′-GGG CAG AAT CAT CAC GAA GT-3′	78	101
	5′-TCT CCT CAT GCT AGT AGC TGC CTT-3′		
CXCR4	5′- GCT TCC TCG CAG TGT AAG AAA AG-3′	80	71
	5′-TGT TGG CTG AAA AGG TGG TC -3′		
	5′- AAA GAT GTC GGG AAT AGT C-3′		

3.2.5 Análise dos dados

Todos os dados obtidos foram analisados utilizando-se o SPSS (SPSS Inc., version 15.0, Chicago, IL,USA). O dados foram submetidos ao teste de Shapiro-Wilk para verificar a sua normalidade. O teste de Wilcoxon foi utilizado para determinar diferenças estatísticas ($p<0,05$).

3.2.6 Aspectos éticos

Foram observados os aspectos éticos da Resolução 196/96, sendo a pesquisa aprovada pelo Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais (CAAE: 54829414.7.0000.5149/ parecer:1.569.493).

ARTIGOS CIENTÍFICOS

Artigo 1: The need for endodontic treatment and systemic characteristics of hematopoietic stem cell transplantation patients

BOR.2017-0068 – Original Research - Immunology

The need for endodontic treatment and systemic characteristics of hematopoietic stem cell transplantation patients

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Running Head: Endodontic infections among HSCT recipients

Abstract: The aim of this study is to investigate the relationship between the epidemiological and clinical profiles of patients before and after hematopoietic stem cell transplantation (HSCT) and the need for endodontic treatment. Subjects included 188 individuals enrolled in the dental care program for transplanted patients of the School of Dentistry, Federal University of Minas Gerais (Faculdade de Odontologia da Universidade Federal de Minas Gerais, FO-UFMG), attended from March 2011 through March 2016. The patients were subjected to an HSCT conditioning dental regimen based on a thorough clinical and radiographic evaluation. Intraoral periapical and bite-wing X-Ray were asked and after evaluation, specific dental treatment was planned and executed. Demographic and clinical data were collected from the patients' medical records: age, gender, transplantation stage, primary disease, transplant type, medication in use, complete blood count at the time of visit and, need for endodontic treatment. The Kolmogorov-Smirnov and the chi-square tests were used. Leukemia (31.3%) and multiple myeloma (17.9%) were the most prevalent primary diseases. Most patients were subjected to allogeneic-related transplantation (83.6%). Most patients exhibited platelet counts and hemoglobin concentrations below the reference values in the pre-transplantation stage, while the neutrophil and platelet counts and the hemoglobin levels were within the reference ranges in the post-transplantation stage. The proportions of individuals needing endodontic treatment were similar between the pre- and post-transplantation groups: 24.3% and 24.7%, respectively. The systemic conditions of the patients referred for dental treatment were compromised.

Keywords: Immunosuppression; Hematopoietic stem cell transplantation; Endodontics.

Introduction

Dental caries and periapical disease are the two most common pathological conditions that affect the mouth; both might be associated with severe systemic complications.[1] Bacterial contamination of the dental pulp might cause its destruction and the consequent development of periapical lesions,[2] which represent a potential site for dissemination of infection. More than 80% of patients subjected to hematopoietic stem cell transplantation (HSCT) develop at least one episode of infection, and 40% of deaths are due to complications from infection alone or following graft rejection. Approximately 55% of post-transplantation infections are caused by bacteria or viruses and 15% to 30% are caused by fungi.[3] The occurrence of infection depends on the patient's immune response and degree of immunosuppression.[4]

HSCT is widely performed for the treatment of malignant blood disorders, including acute and chronic leukemia, aplastic anemia, myelodysplastic syndromes, severe combined immunodeficiency, lymphoma and some solid tumors, such as breast cancer. The prevalence of oral complications among autologous and allogeneic HSCT recipients is high, the most common being mucositis, xerostomia, palate disorders, graft versus host disease (GVHD) and infection. Complications are associated with substantial increase in morbidity, with significant impairment of patient´s quality of life even many years after transplantation.[5] Complications derived from root canal infections might occur at any stage of the transplantation process and can cause significant problems, such as systemic infection or other disorders, which increase the cost and mortality rate associated with transplantation.[6] While in autologous HSCT transplantation most of these problems become minimized six months after the procedure, patients subjected to allogeneic transplantation might develop GVHD-related complications subsequently.[5]

Prevention of systemic complications demands achieving stabilization or elimination of oral infection before the onset of transplantation or myelosuppressive therapy.[6] Additionally, the possible late consequences of total body irradiation and high-dose chemotherapy in immunosuppressed patients are a cause of much concern.[7]

Global care of patients subjected to HSCT also includes routine dental assessments within a multi-professional context. The aim of the present study was to investigate the relationship between the epidemiological and clinical profiles of patients before and after HSCT and the need for endodontic treatment.

Methods

Patients

The study population consisted of individuals enrolled in the dental care program for transplanted patients of the School of Dentistry, Federal University of Minas Gerais (Faculdade de Odontologia da Universidade Federal de Minas Gerais, FO-UFMG). These patients were referred by the HSCT service, Clinical Hospital, UFMG (Hospital de Clínicas, HC-UFMG), from March 2011 through March 2016. Patients whose medical records could not be retrieved were excluded from the study. The patients were subjected to an HSCT conditioning dental regimen based on a specific protocol applied at the HSCT Unit, HC-UFMG. The conditioning dental regimen consists in a thorough clinical and radiographic evaluation of the patient. Intraoral periapical and bite-wing X-Ray, associated with panoramic radiograph were asked. The criteria adopted to determine the need for endodontic treatment were based on clinical and radiographic analyses, along with pulp vitality tests. After further evaluation, specific dental treatment was planned and executed taking in consideration time available before transplant and patient systemic condition. The patients also received dental care after transplantation to maintain their oral health.

Personal data and clinical characteristics

Demographic and clinical data were collected from the patients' medical records and included the following: age, gender, transplantation stage, primary disease, transplant type, medication in use, complete blood count at the time of visit, need for endodontic treatment and number of endodontic treatments performed during conditioning dental regimen. Missing or incomplete data were registered as "Missing/omitted data".

Statistical analysis

In the descriptive analysis, the quantitative variables are expressed as means and standard deviations. The Kolmogorov-Smirnov test was used to investigate whether the data had a normal distribution, and the chi-square test was employed to establish whether there were statistically significant associations between variables. Statistical significance was defined as a p-value of 0.05 or less.

Ethics issues

The present study complied with the ethics requirements described in Health Ministry Resolution no. 196/96 and was approved by the research ethics committee of UFMG (CAAE: 54829414.7.0000.5149/ruling:1.569.493; CAAAE: Certificado de Apresentação para Apreciação Ética/Certificate of Presentation for Ethical Appraisal).

Results

Patients' characteristics

A total of 188 individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG, from March 2011 through March 2016, were included in the study; 60.6% were male, and 39.4% were female. The participants' ages varied from 06 to 69 years old. A total of 103 patients were in the pre-transplantation stage, and 85 were in the post-transplantation stage. Most were allogeneic-related transplants (83.6%), while allogeneic-unrelated transplants corresponded to 9.6% and autologous transplants to 6.8% (TABLE 1). There was no statistically significant association between gender and donor type ($p = 0.57$). The median time from diagnosis to transplantation was 12 months.

Need for endodontic treatment

The frequencies of endodontic treatment were 24.3% and 24.7% before and after HSCT, respectively, corresponding to 23.2% of the targeted sample. There was no statistically significant difference between the groups ($p > 0.05$). Most patients needed endodontic treatment for more than one tooth.

Table1: Description of the patients' characteristics

		Frequency	%
Patients	Before HSCT	103	54.8
	After HSCT	85	45.2
	Total	188	100
Gender	Male	114	60.6
	Female	74	39.4
	Total	188	100
Transplant type	Allogeneic related	61	83.6
	Allogeneic unrelated	7	9.6
	Autologous	5	6.8
	Total	73	100
	Missing	12	

Systemic disease that led to HSCT

Leukemia corresponded to 31.3% of the cases and was the predominant condition among individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG, both before and after HSCT. Acute myeloid leukemia (AML) was exhibited by 15.7% of the sample, chronic myeloid leukemia (CML) by 12.4%, acute lymphocytic leukemia (ALL) by 2.7% and chronic lymphocytic leukemia (CLL) by 0.5%. Approximately 15.1% of the patients had bone marrow aplasia, and 17.9% had multiple myeloma (TABLE 2).

Leukemia was also the main primary disease among transplanted individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG, 44.1%, with 22.6% cases of AML, 16.7% cases of CML, and 4.8% cases of ALL. Approximately 22.6% of the patients had bone marrow aplasia and 8.3% had myelodysplastic syndromes as the primary disorder.

Hematologic analysis before and after HSCT

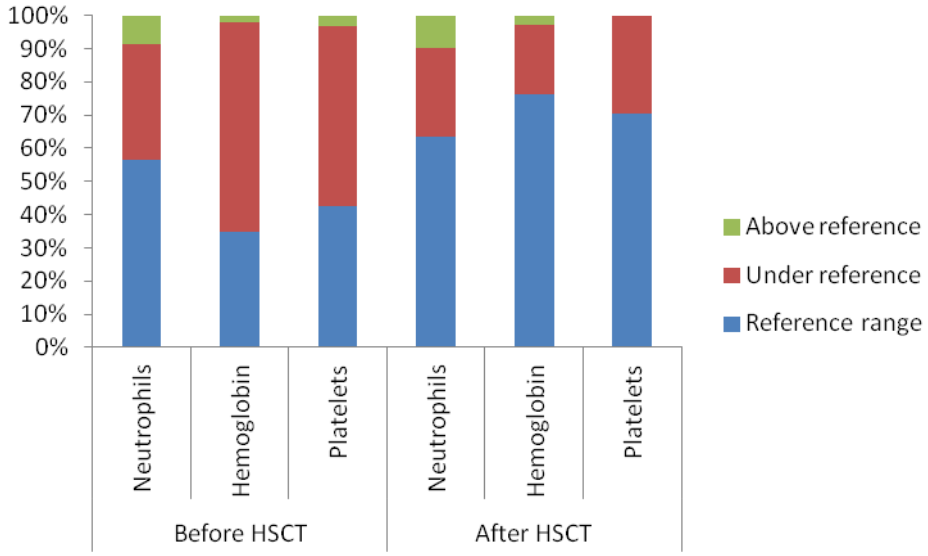
In most patients (56.5%), the neutrophil count was within the reference range before HSCT. However, the platelet count and hemoglobin concentration were below the reference values in 54.3% and 63% of the sample, respectively. There were no statistically significant relationships between gender ($p > 0.05$) and neutrophil and platelet counts and hemoglobin concentration.

In most individuals in the Program of Dental Care for Transplanted Patients (FO-UFMG), after HSCT, the neutrophil and platelet counts and hemoglobin concentration were within the reference ranges, i.e., 64.4%, 70.4% and 76.1% of the sample, respectively. There were no statistically significant relationships between gender and neutrophil and platelet counts and hemoglobin concentration ($p > 0.05$) (Figure 1).

Table2: Percentages of primary diseases exhibited by individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG

Primary disease	Patient		
	Global	Before HSCT	After HSCT
Leukemia	31.3%	20.8%	44.1%
CML	12.4%	8.9%	16.7%
AML	15.7%	9.9%	22.6%
ALL	2.7%	1.0%	4.8%
CLL	0.5%	1.0%	0.0%
Multiple myeloma	17.9%	27.7%	6.0%
Myelodysplastic syndrome	4.9%	2.0%	8.3%
Bone marrow aplasia	15.1%	8.9%	22.6%
Non-Hodgkin's lymphoma	4.3%	5.0%	3.6%
Hodgkin's lymphoma	4.3%	5.9%	2.4%
Other	22.2%	29.7%	13.1%
Total	100%	100%	100%

Figure 1: Complete blood results of individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG



Prescribed medications and percentages of patients using medication before and after HSCT

Most of the analyzed patients (75.5%) used some medication.

After HSCT, 52.9% of the patients used immunosuppressive agents, and 51.8% of them used antibiotics, with cyclosporine and Bactrim® used as the first choices, respectively.

A total of 23.3% of the patients in the pre-transplantation stage used anticancer drugs, and 13.6% used bisphosphonate. Antihypertensive drugs were continuously used by 20.4% and 25.9% of the patients before and after HSCT, respectively. Steroids were used by 22.3% and 18.8% of the pre- and post-transplantation patients, respectively (TABLE 3).

Table 3: Percentages of medications used by individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG

Medication	Patients		
	Global	Before HSCT	After HSCT
In use	75.5%	75.7%	75.3%
Immunosuppressant	23.9%	0.0%	52.9%
Tacrolimus	5.3%	0.0%	11.8%
Cyclosporine	18.6%	0.0%	41.2%
Steroids	20.7%	22.3%	18.8%
Opioid analgesics	4.8%	6.8%	2.4%
Antibiotics	41.0%	32%	51.8%
Bactrim®	36.8%	27.2%	48.2%
Penicillin	4.8%	3.9%	5.9%
Clindamycin	0.5%	0.0%	1.2%
Cephalosporin	0.5%	1.0%	0.0%
Tetracycline	0.5%	1.0%	0.0%
Norfloxacin	2.0%	1.0%	3.5%
Antifungal	1.0%	1.9%	0.0%
Acyclovir	1.0%	1.9%	0.0%
Antihypertensives	22.9%	20.4%	25.9%
Nifedipine/Amlodipine	8.5%	1.9%	16.5%
Atenolol/Propranolol/Metoprolol	5.3%	5.8%	4.7%
Losartan	6.4%	7.8%	4.7%
Captopril/Enalapril	6.4%	5.8%	7.1%
Antineoplastic	13.8%	23.3%	2.4%
Cyclophosphamide	7.0%	11.7%	1.2%
Hydroxyurea	2.0%	2.9%	1.2%
Dasatinib/Sprycel®	2.0%	3.9%	0.0%
Imatinib	1.5%	2.9%	0.0%
Other	1.5%	3.9%	0.0%
Bisphosphonate	9.6%	13.6%	4.7%
Diuretics	4.8%	6.8%	2.4%
Hydrochlorothiazide	2.0%	3.9%	0.0%
Furosemide	1.5%	2.9%	0.0%
Spironolactone	1.5%	1.0%	2.4%
Antacid	35.7%	22.3%	51.8%
Hormone supplements	5.3%	1.0%	10.6%
Vitamin complex	3.7%	4.9%	2.4%
Anticoagulant	9.6%	16.5%	1.2%
Antidepressant	7.0%	7.8%	5.9%

Discussion

Apical periodontitis is an inflammatory disease that affects the tissues surrounding the apical portion of the dental root and is primarily caused by microorganisms infecting the root canal. It represents a potential site for dissemination of infection.[2] Root canal infections among autologous and allogeneic HSCT recipients can be associated with substantial increase in morbidity, with significant impairment of patient´s quality of life.[5] This study assayed the need for endodontic treatment in patients before and after HSCT, analyzing their systemic data and correlating them with the risk that HSCT recipients are submitted in case of persisting endodontic infections.

Autologous HSCT is used for the treatment of malignant conditions, such as multiple myeloma and Hodgkin's and non-Hodgkin's lymphoma. Allogeneic transplantation is often the first-choice treatment for several malignant blood diseases, such as AML, CML, ALL, CLL and severe aplastic anemia. In the present study, almost all of the participants with the above mentioned conditions were subjected to allogeneic HSCT (83.6%). In addition, a retrospective cohort study conducted in Brazil found that most (72%) among 731 patients subjected to HSCT for the treatment of AML received allogeneic transplants.[8] However, these findings disagree with other reports in the literature. One study performed in Spain found that among 228 patients subjected to HSCT, 55.7% received autologous transplants and 44.3% received allogeneic transplants.[9] Another study analyzed data from 1516 transplant centers of 75 countries and demonstrated the largest proportion of patients received autologous transplants (58%).[10] The mortality rate is lower for autologous compared to allogeneic transplantation, with the 5-year mortality associated with allogeneic transplantation varying from 24% to 34%.[11,12]

Interestingly, most of the patients analyzed in the present study were male. This finding disagrees with findings corresponding to individuals infected with human immunodeficiency virus (HIV) and subjected to highly active antiretroviral therapy (HAART) and patients with aplastic anemia, most of whom are female (57.2% and 56.5%, respectively).[13,14] The median time from diagnosis to transplantation was rather long, approximately 12 months. In one retrospective study conducted in Porto Alegre, Rio Grande do Sul (RS), Brazil,

in 2013,[15] the time to transplantation was less than 12 months for the majority (62.9%) of patients (n = 278) subjected to allogeneic HSCT.

Leukemia was the main reason for the analyzed population to be included in the study groups, i.e., before or after HSCT. In other studies, leukemia was also the main primary malignant disease that led to transplantation.[9,10,15,16] The fact that only 6% of the 84 patients subjected to transplantation had multiple myeloma is noteworthy. The reason for such a low prevalence of this condition is that the odds of a cure are very low, and patients exhibit poor survival rates.[17,18] In contrast, another study reported a larger proportion of patients with multiple myeloma (20.15%) among 137 individuals subjected to HSCT.[19]

As a rule, patients subjected to HSCT exhibit pancytopenia before and immediately after transplantation and remain in a state of neutropenia for approximately 6 to 12 months after the procedure. Root canal infections might have serious consequences during this neutropenic period and can eventually compromise graft success.[20] The high risk of bacterial infection after HSCT is due to severe neutropenia and the damage to the body barriers caused by the conditioning regime.[21] In our study, only 56.5% of the patients in the pre-transplantation stage exhibited neutrophil counts within the reference range; this proportion was lower compared to the transplanted patients (63.4%). In addition, most of the patients in the pre-transplantation stage had platelet counts and hemoglobin concentrations below the reference values.

HSCT induces a state of immunosuppression, made even worse by various medications, as shown in the present study. Approximately 20.74% of the patients used steroids, which have strong effects on the distribution and function of neutrophils, monocytes and lymphocytes. In cancer patients, steroids seldom are the only class of immunosuppressive drugs prescribed; therefore, it is difficult to assess their impact on the immune system. The risk of infection is associated with the dose and duration of treatment, the degree of neutropenia and the use of immunosuppressive agents (https://www.nccn.org/professional/physician_gls/PDF/infections.pdf).

Prophylactic antibiotic therapy is commonly indicated after HSCT. In our study, 51.8% of the transplanted patients used antibiotics. The NCCN (National

Comprehensive Cancer Network) guidelines classify cancer patients as at low, medium or high risk for infection based on factors such as primary disease, duration of neutropenia, first exposure to chemotherapy, degree of disease activity and intensity of immunosuppressive therapy. Antimicrobial prophylaxis should be considered for individuals at medium or high risk of infection. Fluoroquinolones are the antibiotics indicated to patients with chemotherapy-induced neutropenia and significantly reduce the incidence of infection with Gram-negative bacteria. Antibiotic prophylaxis against pneumococcal infection is indicated for patients with low immunoglobulin (Ig)G levels and chronic GVHD. Antibiotic prophylaxis must also be prescribed to patients vaccinated against pneumococcal disease and should last at least one year following HSCT.[22]

HSCT makes patients more susceptible to infection, as the oral cavity is a relevant source of pathogens likely to cause systemic disorders in this population.[5] It is believed that infections of oral origin occur in approximately 80% of cases.[11] Pulpal and periradicular diseases usually result from direct or indirect involvement of microorganisms present in the oral cavity.[23,24,25] Changes might occur in the oral microbiota before and after chemotherapy.[26] One cohort study found significant increases of oral colonization by opportunistic pathogens, such as *Enterococcus faecalis* and *Candida* spp., among individuals subjected to allogeneic transplantation.[27] Medically relevant microorganisms, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli, Streptococcus sanguinis* and *Prevotella intermedia* [28,29,30,31] have been detected in infected root canal systems, which highlights the need for endodontic treatment, especially in the case of immunosuppressed patients, such as the patients analyzed in the present study.

Analyses of the need for endodontic treatment of a given population are difficult to find in the international literature;[13,14] the same applies to cases of patients subjected to or to being subjected to HSCT. In the present study, 23.2% of the patients required endodontic intervention. This rate seems quite high when compared to those found for Brazil as a whole within the context of the Health Ministry “SB Brasil” (Oral Health, Saúde Bucal – SB) program, which were 6.2% and 4.3% for the age ranges 15 to 19 and 35 to 44 years old,

respectively. Rates varying from 1.8% to 13% were published for adolescents in Lithuania and Manhattan (USA), respectively.[32,33] The rates found in the present study are also somewhat higher than those obtained for individuals with sickle cell anemia (10.2%)[14] and HIV-seropositivity under HAART (14%),[13] a fact that is fully in agreement with the severe degree of immunosuppression exhibited by patients subjected to HSCT. Although HSCT is crucial for the improvement and survival of patients, infection after transplantation is a relevant cause of morbidity and mortality. Countless factors determine the success or failure of this type of systemic intervention.

Conclusion

The systemic conditions of the patients referred for dental treatment were compromised, especially in the pre-transplantation stage, and were associated with a high prevalence of the need for endodontic treatment. In the last instance, these findings show that when untreated, root canal infections will unequivocally compromise attempts at ensuring global health for this population of patients, along with HSCT itself. Finally, the present study incisively seeks to bring the need for consistent interdisciplinary analysis into debate in the various fields of knowledge to attain increasingly more satisfactory and substantial results in the attempt at ensuring global health to patients requiring stem cell transplantation.

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Reference

1 Overholser CD, Peterson DE, Williams LT, Schimpff SC. Periodontal infection in patients with acute nonlymphocyte leukemia. Prevalence of acute exacerbations. Arch Intern Med. 1982;142(3):551-4.

2 Takahashi K. Microbiological, pathological, inflammatory, immunological and molecular biological aspects of periradicular disease. Int Endod J. 1998;31(5):311-25.

3 Eun SC. Composite tissue allotransplantation immunology. Arch Plast Surg. 2013;40(2):141-53.

4 Nappalli D, Lingappa A. Oral manifestations in transplant patients. Dent Res J (Isfahan). 2015;12(3):199-208.

5 Haverman TM, Raber-Durlacher JE, Rademacher WM, Vokurka S, Epstein JB, Huisman C, et al. Oral complications in hematopoietic stem cell recipients: the role of inflammation. Mediators Inflamm. 2014;2014:378281:1-18.

6 Forman SJ, Blume KG, Thomas ED. Hematopoietic cell transplantation: Blackwell Science; 1999.

7 Curtis RE, Rowlings PA, Deeg HJ, Shriner DA, Socie G, Travis LB, et al. Solid cancers after bone marrow transplantation. N Engl J Med. 1997;336(13):897-904.

8 Lamego RM, Clementino NCD, Costa ÂLB, Oliveira MJM, Bittencourt H. Transplante alogênico de células-tronco hematopoéticas em leucemias agudas: a experiência de dez anos do Hospital das Clínicas da UFMG. Revista Brasileira de Hematologia e Hemoterapia. 2010;32:108-15.

9 Corcia Palomo Y, Knight Asorey T, Espigado I, Martin Villen L, Garnacho Montero J. Mortality of Oncohematological Patients Undergoing Hematopoietic Stem Cell Transplantation Admitted to the Intensive Care Unit. Transplant Proc. 2015;47(9):2665-6.

10 Gratwohl A, Pasquini MC, Aljurf M, Atsuta Y, Baldomero H, Foeken L, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. Lancet Haematol. 2015;2(3):91-100.

11 Greinix HT, Nachbaur D, Krieger O, Eibl M, Knobl P, Kalhs P, et al. Factors affecting long-term outcome after allogeneic haematopoietic stem cell transplantation for acute myelogenous leukaemia: a retrospective study of 172 adult patients reported to the Austrian Stem Cell Transplantation Registry. Br J Haematol. 2002;117(4):914-23.

12 Doney K, Hagglund H, Leisenring W, Chauncey T, Appelbaum FR, Storb R. Predictive factors for outcome of allogeneic hematopoietic cell transplantation for adult acute lymphoblastic leukemia. Biol Blood Marrow Transplant. 2003;9(7):472-81.

13 de Brito LC, da Rosa MA, Lopes VS, e Ferreira EF, Vieira LQ, Sobrinho AP. Brazilian HIV-infected population: assessment of the needs of endodontic treatment in the post-highly active antiretroviral therapy era. J Endod. 2009;35(9):1178-81.

14 Ferreira SB, Tavares WL, Rosa MA, Brito LC, Vieira LQ, Martelli HJ, et al. Sickle cell anemia in Brazil: personal, medical and endodontic patterns. Braz Oral Res. 2016;30(1):e60.

15 Pitombeira BS, Paz A, Pezzi A, Amorin B, Valim V, Laureano A, et al. Validation of the EBMT Risk Score for South Brazilian Patients Submitted to Allogeneic Hematopoietic Stem Cell Transplantation. Bone Marrow Res. 2013;2013:565824:1-7.

16 Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, et al. Hematopoietic stem cell transplantation: a global perspective. Jama. 2010;303(16):1617-24.

17 Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78(1):21-33.

18 Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, et al. Cancer statistics, 2004. CA Cancer J Clin. 2004;54(1):8-29.

19 Barba P, Burns LJ, Litzow MR, Juckett MB, Komanduri KV, Lee SJ, et al. Success of an International Learning Health Care System in Hematopoietic Cell Transplantation: The American Society of Blood and Marrow Transplantation Clinical Case Forum. Biol Blood Marrow Transplant. 2016;22(3):564-70.

20 Bishay N, Petrikowski CG, Maxymiw WG, Lee L, Wood RE. Optimum dental radiography in bone marrow transplant patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;87(3):375-9.

21 Sviland L, Pearson AD, Green MA, Baker BD, Eastham EJ, Reid MM, et al. Immunopathology of early graft-versus-host disease--a prospective study of skin, rectum, and peripheral blood in allogeneic and autologous bone marrow transplant recipients. Transplantation. 1991;52(6):1029-36.

22 Recommendations of the Center for International Blood and Marrow Transplant Research tNMDPtEBaMTGtASoBaMT, Tomblyn M, Chiller T, Einsele H, Gress R, Sepkowitz K, et al. Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplant Recipients: A Global Perspective. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 2009;15(10):1143-238.

23 Fabricius L, Dahlen G, Holm SE, Moller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res. 1982;90(3):200-6.

24 Moller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr. 1966;74(5):Suppl:1-380.

25 Sundqvist GK, Eckerbom MI, Larsson AP, Sjogren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infect Immun. 1979;25(2):685-93.

26 Napenas JJ, Brennan MT, Coleman S, Kent ML, Noll J, Frenette G, et al. Molecular methodology to assess the impact of cancer chemotherapy on the oral bacterial flora: a pilot study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010;109(4):554-60.

27 Vokurka S, Skardova J, Hruskova R, Kabatova-Maxova K, Svoboda T, Bystricka E, et al. The effect of polyvinylpyrrolidone-sodium hyaluronate gel (Gelclair) on oral microbial colonization and pain control compared with other rinsing solutions in patients with oral mucositis after allogeneic stem cells transplantation. Med Sci Monit. 2011;17(10):Cr572-6.

28 Henriques LC, de Brito LC, Tavares WL, Teles RP, Vieira LQ, Teles FR, et al. Microbial Ecosystem Analysis in Root Canal Infections Refractory to Endodontic Treatment. J Endod. 2016; 42(8):1239-45.

29 Brito LC, Teles FR, Teles RP, Franca EC, Ribeiro-Sobrinho AP, Haffajee AD, et al. Use of multiple-displacement amplification and checkerboard DNA-DNA hybridization to examine the microbiota of endodontic infections. J Clin Microbiol. 2007;45(9):3039-49.

30 Tavares WL, Neves de Brito LC, Teles RP, Massara ML, Ribeiro Sobrinho AP, Haffajee AD, et al. Microbiota of deciduous endodontic infections analysed by MDA and Checkerboard DNA-DNA hybridization. Int Endod J. 2011;44(3):225-35.

31 Siqueira JF, Jr., Alves FR, Rocas IN. Pyrosequencing analysis of the apical root canal microbiota. J Endod. 2011;37(11):1499-503.

32 Brukiene V, Aleksejuniene J, Balciuniene I. Dental treatment needs in Lithuanian adolescents. Stomatologija. 2005;7(1):11-5.

33 Mitchell DA, Ahluwalia KP, Albert DA, Zabos GP, Findley SE, Trinh-Shevrin CB, et al. Dental caries experience in northern Manhattan adolescents. J Public Health Dent. 2003;63(3):189-94.

Artigo 2: The need for endodontic treatment and systemic characteristics of Brazilian Liver Transplant Candidates

The need for endodontic treatment and systemic characteristics of Brazilian Liver Transplant Candidates

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Abstract

Dental caries are the major cause of pulpal inflammation and infection. The outcome of pulpal insult is a dynamic process that depends on both the invading microorganisms and host responses to these agents, which include inflammation and immunity. Increased susceptibility to infections may be related to compromised immune system function in liver transplant candidates. Untreated oral diseases in these patients can lead to infections and sepsis and may cause many complications in transplanted patients. The majority of liver transplants are performed for complications resulting from viral hepatitis or alcoholic cirrhosis. Infections are a frequent cause of morbidity and mortality among these patients. Hence, a prerequisite dental evaluation is usually recommended for potential organ transplant candidates. Studies correlating the need for endodontic treatment with systemic disorders leading to liver transplantation (LT) are scarce in the literature. The present study included 120 liver transplant candidates and correlated their personal and systemic characteristics with the need for endodontic treatment. The need for endodontic treatment in this cohort was 20.7%. Cirrhosis (45%) and viral hepatitis (25.5%) were the most prevalent primary diseases. Approximately 2.0% of the patients had hepatocellular carcinoma. Most patients exhibited platelet counts below the reference values. The lymphocyte counts and haemoglobin concentrations were also below the reference values in 43.3% and 38.3% of the sample, respectively.

Keywords: Endodontic treatment, liver disease, liver transplant, microbiology, immunology, epidemiology.

Introduction

Infections of dental pulp occur as a sequel of caries, unsuccessful operative procedures, and trauma. The pulpal infection is comprised of a polymicrobial community that predominantly contains gram-negative anaerobes. Once bacteria invade and colonize the dentin, the microbes survive within a niche in the root canal system. If appropriate endodontic treatment is not provided, the infected root canal remains a stable source of bacteria (1). The goal of root canal therapy is to prevent or eliminate infections inside the root canal system that have the potential to induce apical periodontitis. To achieve this goal, extirpation and debridement of pulpal tissue and complete obturation of the prepared root canal system are required (2). Moreover, it has been shown that after root canal filling, microorganisms may remain in the periapical tissues (3), and in this case, the immune system is the key element for eliminating the remaining bacteria and healing the periapical tissue.

The association between systemic and oral health is under extensive investigation. Dental diseases have been found to be a risk factor for many systemic diseases and conditions such as diabetes, cardiovascular disease, respiratory disease, chronic kidney disease, and preterm birth (4-6). Infected teeth are thought to add to the overall systemic inflammatory burden in the body and release oral bacterial metabolites into the bloodstream (7).

Liver diseases are a very common health matter, and the main underlying causes include infections, alcohol abuse and lipid and carbohydrate metabolic disorders. Liver failure occurs when the liver loses its ability to function gradually over the course of years or rapidly within days. If the damage is irreversible, liver transplantation is the only solution (8). It has been estimated that 60 to 80% of liver transplant recipients develop an infection, and infection can compromise the survival of any organ transplant recipient. Patients with chronic liver disease (CLD) are susceptible to bacterial infections, which may lead to bacteraemia and eventually to death (9). Increased susceptibility to infections may be related to compromised immune system function in such patients (10).

To prevent such occurrences and to reduce the potential morbidity posed by dental infections, most transplant centres recommend an oral examination as

part of the pretransplant evaluation process aiming to detect and eliminate possible oral infection foci to reduce bacteraemia and eventual morbidity (11).The aim of the present study was to investigate the relationship between the epidemiological and clinical profiles of liver transplant candidates (LTC).

Methods

Patients

The study population consisted of individuals enrolled in the dental care programme for transplanted patients at the School of Dentistry, Federal University of Minas Gerais (Faculdade de Odontologia da Universidade Federal de Minas Gerais, FO-UFMG). These patients were referred by the Liver Transplant service, Clinical Hospital, UFMG (Hospital de Clínicas, HC-UFMG), from March 2012 through March 2016. Patients whose medical records could not be retrieved were excluded from the study. The patients were subjected to a conditioning dental regimen based on a specific protocol applied at the liver transplant unit, HC-UFMG. The patients also received dental care after transplantation to maintain their oral health.

Personal data and clinical characteristics

Demographic and clinical data were collected from the patients' medical records and included the following: age, gender, primary disease, medication in use, complete blood count at the time of visit, need for endodontic treatment and number of endodontic treatments performed. Missing or incomplete data were registered as "Missing/omitted data".

Statistical analysis

In the descriptive analysis, the quantitative variables are expressed as the means and standard deviations. The Kolmogorov-Smirnov test was used to investigate whether the data were normally distributed, and the chi-square test was employed to establish whether there were statistically significant associations between variables.

Ethics issues

The present study complied with the ethics requirements described in Health Ministry Resolution no. 196/96 and was approved by the research ethics committee of UFMG (CAAE: 54829414.7.0000.5149/ruling:1.569.493; CAAAE:

Certificado de Apresentação para Apreciação Ética/Certificate of Presentation for Ethical Appraisal).

Results

Patients' characteristics

A total of 120 individuals who enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG, from March 2012 through March 2016 were included in the study; 72.2% were male, and 27.8% were female. The participants' ages varied from 2 to 73 years old. The median time from diagnosis to transplantation was 32.5 months.

Systemic diseases that led to the need for liver transplant

Cirrhosis was the main primary disease among individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG, and was responsible for 45% of the total cases. Viral Hepatitis was associated with 25.5% of patients, including 23.2% with Hepatitis C and 3.3% with hepatitis B. Approximately 2.0% of the patients had hepatocellular carcinoma (TABLE 1).

Table 1: Description of the patients' characteristics

		Frequency	%
Gender	Male	87	72.5
	Female	33	27.5
	total	120	100
Primary Indication	Cirrhosis	59	49.2
	Hepatitis C	25	20.8
	Hepatitis B	2	1.7
	Autoimmune Hepatitis	4	3.3
	Hepatocellular carcinoma	3	2.5
	Colangite esclerosante	7	5.8
	Other	15	12.5
	Missing	5	4.2
	Total	120	100

Need for endodontic treatment

The need for endodontic treatment was 20.7%. Most of the patients needed endodontic treatment in only one dental tooth.

Prescribed medications and percentages of patients using medication

Most of the analysed patients (92.5%) used some medication.

A total of 90.8% of the patients used diuretics, with Hydrochlorothiazide as the first choice. Antihypertensive drugs were continuously used by 50.8% and antacids were used by 48.3% of the patients (TABLE 2).

Haematologic analysis

In the most individuals (87.4%), the platelet count was below the reference value (<150,000/lL). The lymphocyte count and haemoglobin concentration were also below the reference values in 50.5% and 44.7% of the sample, respectively.

In most individuals enrolled at the Program of Dental Care for Transplanted Patients, FO-UFMG, the basophil, monocyte, eosinophil and neutrophil counts were within the reference ranges in 98%, 79.4%, 67% and 55% of the sample, respectively (FIGURE 1).

Table 2: Percentages of medications used by individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG

Medication	Patients	
	Frequency	%
In use	111	92.5
Immunosuppressant	25	20.9
Tacrolimus	12	10.0
Imunen®	13	10.8
Antacid	58	48.3
Antibiotics	23	19.2
Bactrim®	2	1.7
Penicillin	14	11.7
Tetracycline	2	1.7
Norfloxacin	20	16.7
Antihypertensives	61	50.8
Nifedipine/Amlodipine	9	7.5
Atenolol/Propranolol/Metoprolol	54	45.0
Losartan	2	1.7
Captopril/Enalapril	3	2.5
Diuretics	109	90.8
Hydrochlorothiazide	78	65.0
Furosemide	4	3.3
Spironolactone	60	50.0
Steroids	1	0.8
Laxatives	26	22.0
Vitamin complex	3	2.5
Anticoagulant	4	3.3
Antidepressant	12	10.0

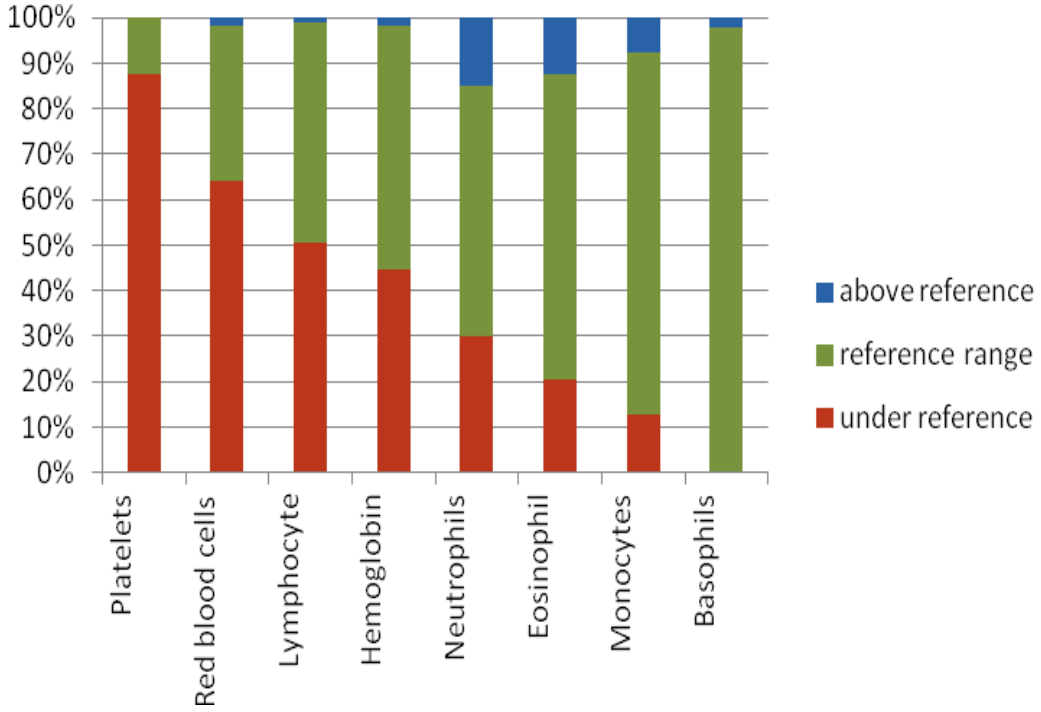

Figure 1: Complete blood results of individuals enrolled in the Program of Dental Care for Transplanted Patients, FO-UFMG

Discussion

The liver has many functions, including building proteins and other substances used by the body as well as drug metabolism and energy storage. The liver also removes waste products and toxins from the blood. Liver disease can lead to crucial system failure. Liver diseases can be classified as *acute* (characterized by rapid resolution and complete restitution of organ structure and function once the underlying cause has been eliminated) or *chronic* (characterized by persistent damage, with progressive organ function impairment followed by liver cell damage. Liver diseases can also be classified as *infectious* (hepatitis A, B, C, D and E viruses, infectious mononucleosis, or secondary syphilis and tuberculosis) or *non-infectious* (substance abuse such as alcohol and drugs, e.g., paracetamol, halothane, ketoconazole, methyldopa and methotrexate) (12).

The majority of liver transplants are performed as a consequence of complications from viral hepatitis or alcoholic cirrhosis. Cirrhosis is attributable

to numerous aetiologies that fall into several broad categories, including infectious (typically viral), toxicologic, immunologic (including autoimmune disease and altered immune response), biliary disease, and obstruction, as well as metabolic and vascular disturbances. The hepatocellular necrosis is a common pathogenic feature of these varied aetiologies of cirrhosis. Cirrhosis was the main reason for the analysed population to be included in the study group (45%). In the United States, an estimated 5.5 million people have chronic liver disease, including cirrhosis. Over 60% of patients are male, and over 80% are between 25 and 64 years of age (13). The results of the present study also show that most of the patients analysed were male (72.2%), and the participants' ages varied from 2 to 73 years old, and the median age was 52.5. The median time from diagnosis to transplantation was rather long, approximately 975 days. According to the Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients (OPTN and SRTR), the average time to transplant in United States was 382 days (14).

Dental caries and periapical disease are the two most common pathological conditions that affect the mouth, and both disorders may be associated with severe systemic complications (15). Bacterial contamination of the dental pulp might cause its destruction and the consequent development of periapical lesions (16), which represent a potential site for dissemination of infection. Patients with CLD, particularly those with hepatitis C virus infection or in those cases where the liver impairment is associated with alcohol abuse, tend to present with deficient oral hygiene (17, 18). A number of features associated with advanced liver disease can diminish the amount of saliva, which in turn promotes the deposition and retention of dental plaque and increases patient vulnerability to caries (17, 19). The management of ascites and/or oedema, which are frequent complications of cirrhosis, may require the use of diuretic agents that can reduce saliva production. In the present study, 90.8% of the LTCs were taking diuretic agents. Guggenheimer et al. 2007 (19) showed that the use of diuretics was significantly associated with the presence of dental plaque. Grossmann et al. (20) observed that many patients with hepatitis C infection present with poor dental health. Other authors also

reported worsened dental conditions in patients with liver cirrhosis (21, 22). In previous studies alcohol and hepatitis C cirrhotic patients had the lowest numbers of teeth when compared with healthy controls (23, 24).

Analyses of the need for endodontic treatment in a given population are difficult to find in the international literature. In the present study, 20.7% of the patients required endodontic intervention. The rates found in the present study are similar to those obtained for individuals undergoing to HSCT (24.3%) (25).The increased need for endodontic treatment may also be related to compromised immune system function in such patients. A recent study reported that LTCs have a significantly higher prevalence of radiographic periapical lesions compared with the control group (26). However, the rates found in the present study seems quite high when compared with those found for Brazil as a whole within the context of the Health Ministry “SB Brasil” (Oral Health, Saúde Bucal – SB) programme, which were 6.2% and 4.3% for the age ranges 15 to 19 and 35 to 44 years old, respectively (www.saude.gov.br/bucal). Other rates varying from 1.8% to 14% have been reported in several other studies (2, 27-29).

Among LTCs, other physical, behavioural, and/or social comorbidities that can contribute to untreated dental disease, as well as tooth loss, include their older age, disability with loss of insurance, preoccupation with medical issues, lack of motivation, anxiety and/or depression, poor health behaviours, or an inability to comply with obligatory health regimens (11). These factors may also impact the results observed here since patients wait for a long time to be transplanted, as demonstrated elsewhere (28).

As noted previously, dental breakdown may increase susceptibility to infections before and after LT. Dental infections should therefore be actively identified and treated accordingly. However, there are several concerns about the risks of performing dental surgical procedures in such frail patients. In this respect, some authors (30-32) have reported several cases of haemorrhagic complications and delayed wound healing in patients with CLD undergoing dental surgery, leading some authorities to contraindicate surgery in those patients (21). Thrombocytopenia (platelet counts <150,000/lL) is a common complication in patients with chronic liver disease (CLD) and has been

observed in as many as 76% of cirrhotic patients. In this study, the majority of the selected population (87.4%) presented platelet counts below the reference value. Severe thrombocytopenia can be associated with significant morbidity, which often complicates the medical management of patients with advanced liver disease (33).

It is well known that neutrophils, as well as the antibody/complement system, are critical for protection against periodontal bacteria (10). Patients with cirrhosis have an increased susceptibility to bacterial infections and have a compromised immune system function (34). In our study, a high percentage of the patients in the pre-transplantation stage exhibited neutrophil counts below the reference range (30.1%). Bacteria typically involved in oral infections, such as *Streptococcus viridans,* are increasingly being recognized as a cause of spontaneous bacterial peritonitis among patients with cirrhosis (35), but a causal relationship has not been established.

Dental infections have been implicated in the pathophysiology of several systemic diseases, including diabetes, cardiovascular disease, respiratory disease, chronic kidney disease, and preterm birth (4-6). Recent studies (36, 37) suggest the real necessity of removing dental infections before transplantation. The results from another study (38) showed that patients who did not have dental treatment prior to stem cell transplantation had considerably more complications soon thereafter. In accordance, a high risk of infections was observed in patients presenting with acute liver failure without pretransplant dental treatment (36). Arvaniti et al. (37) reported that CLD patients are 4-fold more susceptible to mortality after infection due to their immune system impairment. Additionally, a retrospective study found an association between dental infections and accelerated liver diseases (39).

Patients with CLD considered for liver transplantation have to be carefully examined for the presence of dental infection, which should be eliminated before transplantation. This examination is worthwhile since patients will be immunosuppressed as a consequence of the treatment protocol during the post transplantation period (36). As observed here, poor oral health status and odontogenic infections are frequently found among patients with CLD, which may represent a source of systemic infections before and after LT. In an

attempt to reduce post transplantation mortality, multidisciplinary assistance ought to be designed in different ambulatory care units around the world.

References

1. Sasaki H, Hirai K, Martins CM, Furusho H, Battaglino R, Hashimoto K. Interrelationship Between Periapical Lesion and Systemic Metabolic Disorders. Curr Pharm Des. 2016;22(15):2204-15.
2. de Brito LC, da Rosa MA, Lopes VS, e Ferreira EF, Vieira LQ, Sobrinho AP. Brazilian HIV-infected population: assessment of the needs of endodontic treatment in the post-highly active antiretroviral therapy era. J Endod. 2009;35(9):1178-81.
3. Garcia CC, Sempere FV, Diago MP, Bowen EM. The post-endodontic periapical lesion: histologic and etiopathogenic aspects. Med Oral Patol Oral Cir Bucal. 2007;12(8):E585-90.
4. Heimonen A, Janket SJ, Kaaja R, Ackerson LK, Muthukrishnan P, Meurman JH. Oral inflammatory burden and preterm birth. J Periodontol. 2009;80(6):884-91.
5. Scannapieco FA, Dasanayake AP, Chhun N. "Does periodontal therapy reduce the risk for systemic diseases?". Dent Clin North Am. 2010;54(1):163-81.
6. Fisher MA, Borgnakke WS, Taylor GW. Periodontal disease as a risk marker in coronary heart disease and chronic kidney disease. Curr Opin Nephrol Hypertens. 2010;19(6):519-26.
7. Offenbacher S, Barros SP, Beck JD. Rethinking periodontal inflammation. J Periodontol. 2008;79(8 Suppl):1577-84.
8. Radmand R, Schilsky M, Jakab S, Khalaf M, Falace DA. Pre-liver transplant protocols in dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(4):426-30.
9. Caly WR, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. J Hepatol. 1993;18(3):353-8.
10. Anand AC, Pardal PK, Sachdev VP. DENTAL CARIES AND PERIODONTAL DISORDERS IN CHRONIC LIVER DISEASE. Med J Armed Forces India. 2001;57(1):26-30.

11. Guggenheimer J, Eghtesad B, Stock DJ. Dental management of the (solid) organ transplant patient. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;95(4):383-9.
12. Grau-Garcia-Moreno DM. [Dental management of patients with liver disease]. Med Oral. 2003;8(3):231.
13. Vong S, Bell BP. Chronic liver disease mortality in the United States, 1990-1998. Hepatology. 2004;39(2):476-83.
14. Thuluvath PJ, Guidinger MK, Fung JJ, Johnson LB, Rayhill SC, Pelletier SJ. Liver transplantation in the United States, 1999-2008. Am J Transplant. 2010;10(4 Pt 2):1003-19.
15. Overholser CD, Peterson DE, Williams LT, Schimpff SC. Periodontal infection in patients with acute nonlymphocyte leukemia. Prevalence of acute exacerbations. Arch Intern Med. 1982;142(3):551-4.
16. Takahashi K. Microbiological, pathological, inflammatory, immunological and molecular biological aspects of periradicular disease. Int Endod J. 1998;31(5):311-25.
17. Guggenheimer J, Moore PA. Xerostomia: etiology, recognition and treatment. J Am Dent Assoc. 2003;134(1):61-9; quiz 118-9.
18. Coates EA, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, et al. Hepatitis C infection and associated oral health problems. Aust Dent J. 2000;45(2):108-14.
19. Guggenheimer J, Eghtesad B, Close JM, Shay C, Fung JJ. Dental health status of liver transplant candidates. Liver Transpl. 2007;13(2):280-6.
20. Grossmann Sde M, Teixeira R, de Aguiar MC, de Moura MD, do Carmo MA. Oral mucosal conditions in chronic hepatitis C Brazilian patients: a cross-sectional study. J Public Health Dent. 2009;69(3):168-75.
21. Lins L, Bittencourt PL, Evangelista MA, Lins R, Codes L, Cavalcanti AR, et al. Oral health profile of cirrhotic patients awaiting liver transplantation in the Brazilian Northeast. Transplant Proc. 2011;43(4):1319-21.
22. Bagan JV, Alapont L, Sanz C, del Olmo JA, Morcillo E, Cortijo J, et al. [Dental and salivary alterations in patients with liver cirrhosis: a study of 100 cases]. Med Clin (Barc). 1998;111(4):125-8.

23. Guggenheimer J, Mayher D, Eghtesad B. A survey of dental care protocols among US organ transplant centers. Clin Transplant. 2005;19(1):15-8.
24. Piekarczyk J, Fiedor P, Chomicz L, Szubinska D, Starosciak B, Piekarczyk B, et al. Oral cavity as a potential source of infections in recipients with diabetes mellitus. Transplant Proc. 2003;35(6):2207-8.
25. Bambirra W, Jr., Maciel KF, Thebit MM, de Brito LC, Vieira LQ, Sobrinho AP. Assessment of Apical Expression of Alpha-2 Integrin, Heat Shock Protein, and Proinflammatory and Immunoregulatory Cytokines in Response to Endodontic Infection. J Endod. 2015;41(7):1085-90.
26. Castellanos-Cosano L, Machuca-Portillo G, Segura-Sampedro JJ, Torres-Lagares D, Lopez-Lopez J, Velasco-Ortega E, et al. Prevalence of apical periodontitis and frequency of root canal treatments in liver transplant candidates. Med Oral Patol Oral Cir Bucal. 2013;18(5):e773-9.
27. Brukiene V, Aleksejuniene J, Balciuniene I. Dental treatment needs in Lithuanian adolescents. Stomatologija. 2005;7(1):11-5.
28. Mitchell DA, Ahluwalia KP, Albert DA, Zabos GP, Findley SE, Trinh-Shevrin CB, et al. Dental caries experience in northern Manhattan adolescents. J Public Health Dent. 2003;63(3):189-94.
29. Ferreira SB, Tavares WL, Rosa MA, Brito LC, Vieira LQ, Martelli HJ, et al. Sickle cell anemia in Brazil: personal, medical and endodontic patterns. Braz Oral Res. 2016;30(1).
30. Helenius-Hietala J, Aberg F, Meurman JH, Nordin A, Isoniemi H. Oral surgery in liver transplant candidates: a retrospective study on delayed bleeding and other complications. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;121(5):490-5.
31. Perdigao JP, de Almeida PC, Rocha TD, Mota MR, Soares EC, Alves AP, et al. Postoperative bleeding after dental extraction in liver pretransplant patients. J Oral Maxillofac Surg. 2012;70(3):e177-84.
32. Pereira Tdos S, Pelinsari FC, Ruas BM, Avelar LP, da Fonseca VJ, de Abreu MH, et al. Postoperative complications after dental extraction in liver pretransplant patients. Spec Care Dentist. 2016;36(5):277-81.
33. Peck-Radosavljevic M. Thrombocytopenia in chronic liver disease. Liver Int. 2016.

34. Barnes PF, Arevalo C, Chan LS, Wong SF, Reynolds TB. A prospective evaluation of bacteremic patients with chronic liver disease. Hepatology. 1988;8(5):1099-103.
35. Cholongitas E, Papatheodoridis GV, Lahanas A, Xanthaki A, Kontou-Kastellanou C, Archimandritis AJ. Increasing frequency of Gram-positive bacteria in spontaneous bacterial peritonitis. Liver Int. 2005;25(1):57-61.
36. Helenius-Hietala J, Aberg F, Meurman JH, Isoniemi H. Increased infection risk postliver transplant without pretransplant dental treatment. Oral Dis. 2013;19(3):271-8.
37. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010;139(4):1246-56, 56.e1-5.
38. Melkos AB, Massenkeil G, Arnold R, Reichart PA. Dental treatment prior to stem cell transplantation and its influence on the posttransplantation outcome. Clin Oral Investig. 2003;7(2):113-5.
39. Aberg F, Helenius-Hietala J, Meurman J, Isoniemi H. Association between dental infections and the clinical course of chronic liver disease. Hepatol Res. 2014;44(3):349-53.

Artigo 3: Immunological profile of periapical endodontic infections in patients undergoing hematopoietic transplantation

Immunological profile of periapical endodontic infections in patients undergoing hematopoietic transplantation

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Abstract

More than 80% of patients subjected to haematopoietic stem cell transplantation (HSCT) develop at least one episode of infection. Bacterial contamination of the dental pulp might cause its destruction and the consequent development of periapical lesions, which represent potential sites for the dissemination of infection. The aim of this study was to evaluate the mRNA expression levels of cytokines interferon-γ, tumour necrosis factor-α, interleukin IL-1β, IL-10, and the chemokines CCL2/MCP-1, CCL4 and CXCR4 in interstitial fluid from root canal infections. The case group was composed of 10 patients undergoing HSCT, and our control group included 10 healthy patients. Clinical samples were taken from teeth with pulp necrosis. After cleaning and drying, 3 paper points were introduced into the root canal, passing passively through the root apex (2 mm) into the periapical tissues for 1 min. Samples were collected immediately after root canal cleaning and 7 days later (restrained root canal bacterial load) to characterize gene expression using real-time PCR. The results showed significantly increased expression levels of TNF-α and IFN-γ on day 7 in control and case groups. The mRNA levels of IL-1β and IL-10 in the

bone marrow group increased in the samples from day 7. The chemokine CCL-2/MCP-1 was not detected in patients undergoing HSCT. The expression of CCL-4 was not significantly different between the groups. Chemokine receptor CXCR4 levels increased in samples obtained from the day 7 control group. Individuals undergoing HSTC presented proinflammatory and anti-inflammatory action during periapical responses. Similar outcomes were observed between the cases and individuals in the control group.

Keywords: Endodontic treatment, hematopoietic stem cell transplantation, immunosuppression, endodontic infection, cytokines and chemokines

Introduction

Haematopoietic stem cell transplantation (HSCT) is widely used to treat malignant blood disorders, including acute and chronic leukaemia, aplastic anaemia, myelodysplastic syndromes, severe combined immunodeficiency, lymphoma and some solid tumours, such as breast cancer. The prevalence of oral complications among HSCT recipients is high, the most common of these complications being mucositis, xerostomia, palate disorders, graft versus host disease (GVHD) and infection (1). Complications derived from root canal infections might occur at any stage of the transplantation process and can cause significant problems, such as systemic infection or other disorders (2). Propagation of the infection depends on local and systemic host factors and on the virulence of the pathogen (3).

Apical periodontitis is an inflammatory disease of periradicular tissues that is caused by the host immune response to root canal infection and is characterized by localized inflammation concomitant with bone resorption (4). During maturation of the immune response, antigen-presenting cells are responsible for the polarization of T-helper (Th) immune profiles. Four T-cell subsets have been described: Th1, Th2, Th17, and T-regulatory (Treg) cells. Each of these drives a characteristic protective immune response (5).

The inflammatory response is related to a type 1 immune response, which is characterized by the production of interferon- γ (IFN- γ), tumour necrosis factor-α (TNF-α), and interleukin-1 (IL-1), which are involved in the disease progression, bone destruction, and remodelling of periapical lesions (4). In contrast, immunosuppressive mechanisms mediated by Treg-derived or Th2-

derived cytokines are responsible for healing and restricting the inflammatory immune mechanisms (6). Transforming growth factor (TGF)-β and lL-10, which was initially described as a Th2 cytokine, both exhibit strong anti-inflammatory properties.

Chemokines such as CCL2/MCP-1, CXCR4 and CCL4 compose a specialized group of cytokines that coordinate cell movements that are necessary for the initiation of T-cell immune responses and recruiting the appropriate effector cells to sites of inflammation, including Th 1 or Th 2 cells (7). These molecules are involved in many biological processes, including organ development and homeostasis, angiogenesis, and immune activation and regulation (8).

Patients undergoing HSCT are at higher risk of infection. Neutropenia has been recognized as a major risk factor for the development of infections in these patients (9). Although impaired immunologic responses have been shown in HSCT individuals, no study has analysed the periapical immune responses to root canal infections in these individuals. The aim of this study was to quantitatively assay the expression of proinflammatory (IFN-γ, TNF-α, lL-1β), and Treg (IL-10) cytokines and the expression of chemokines (CCL-2/MCP-1, CCL-4, and CXCR4) in samples collected from interstitial fluid adjacent to root canal infections in healthy/control and pre-HSCT/bone marrow group individuals. Seven days later, the same parameters were assayed following root canal cleaning procedures, which significantly reduced root canal bacterial load. The healing process had presumably begun in the second group of samples.

Materials and Methods

Human subjects

Subjects included 10 healthy patients and 10 patients at the pre-HSCT stage who were selected from 103 males and females (children and adults) enrolled in the dental care program for transplanted patients at the School of Dentistry at the Federal University of Minas Gerais (Faculdade de Odontologia da Universidade Federal de Minas Gerais, FO-UFMG). Patients enrolled in the dental care program were referred by the HSCT service, Clinical Hospital, UFMG (Hospital de Clínicas, HC-UFMG), from March 2011 through March 2016. All patients updated their medical records at the first appointment. The patients were subjected to an HSCT-conditioning dental regimen based on a specific protocol applied at the HSCT Unit, HC-UFMG. The conditioning dental regimen comprised a thorough clinical and radiographic evaluation of the patient. Intraoral periapical and bite-wing radiographs combined with panoramic radiographs were requested. The selected patients had teeth with pulp necrosis and apical periodontitis and were between 29 and 61 years old. All participants signed the Free Agreement Formulary. This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (CAAE: 54829414.7.0000.5149/ruling:1.569.493).

Sample collection

Clinical samples were taken from teeth with pulp necrosis, which was diagnosed based on clinical and radiographic analyses and pulp sensibility tests. Teeth did not present acute periapical symptoms at the time of the appointment. The sampling procedures were performed as previously described (3). Each tooth was isolated, and the root canals were cleaned and shaped

using ProTaper universal NiTi files (Dentsply Maillefer, Ballaigues, Switzerland) and 2.5% sodium hypochlorite. The samples were collected immediately after root canal cleaning to characterize the cytokine/chemokine expression profile. After cleaning and drying, three paper points (#20) were introduced into the root canal, passing passively through the root apex (2 mm) into the periapical tissues, where they remained for 1 min. The paper points were cut at 4 mm from the tip, placed into microcentrifuge tubes, and stored at 70 °C. Using this procedure, RNA was extracted from the periapical interstitial fluid. No endodontic dressing was inserted into the root canals. The coronal access cavities of the teeth were restored using a eugenol-based cement. Seven days later (day 7), the teeth were opened, and the periapical interstitial fluid was sampled again to characterize the expression of cytokine/chemokine expression in teeth with restrained root canal bacterial loads. Single and multiple root teeth were included in this study. In teeth with multiple canals, the first (day 0) and second (day 7) samples were collected from the same canal. At this time, no teeth exhibited clinical signs or symptoms, and the root canals were filled using the lateral compaction technique.

Sample preparation

Total RNA was extracted from each sample using TRIzol reagent (GIBCO/BRL Laboratories, Grand Island, NY) as described elsewhere (10-12) and then stored at -70 °C.

Real-time polymerase chain reaction

Complementary DNA was synthesized using 1 µg of RNA and reverse transcription as described previously (13). Primer sequences were designed using PRIMER EXPRESS software (Applied Biosystems, Foster City, CA, USA)

based on nucleotide sequences available in the GenBank database. Real-time PCR assays were performed using PRIMER EXPRESS software (Applied Biosystems). The primer sequences used for the quantitative PCR analysis of IFN-γ, TNF-α, IL-1β, IL-10, CCL4, CXCR4 and MCP-1 mRNA expression are shown in Table 1. PCR was performed under the following standard conditions: a holding stage at 95 °C (10 min); a cycling stage of 40 cycles at 95 °C (15 s) followed by 60 °C (1 min); and a melting curve stage at 95 °C (15 s), 60 °C (1 min) and 95 °C (15 s). A SYBR-Green detection system (Applied Biosystems) was used to visualize primer amplification. Following amplification, melting curve analysis was performed to determine the specificity of the amplified products. The melting curve was obtained from 60 °C to 95 °C, and continuous fluorescence measurements were recorded at every 1% increase in temperature. PCR products with melting temperatures that diverged from those that have been established for standard DNA were considered false positives; for such cases, a null fluorescence value was attributed. Glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization and was assayed with each set of reactions. All samples were assayed in duplicate. Each reaction was performed in a 25-μL volume containing 1μg of cDNA. Sequence Detection System (SDS) Software version 2.4.1 (Applied Biosystems) was used to analyse the data after amplification. The results were obtained as threshold cycle (Ct) values, and the expression levels were calculated using the comparative $2^{-\Delta\Delta C}{}_{T}$ method (14). The results were calculated as the mean value of duplicate assays for each patient, and the mRNA expression levels in all samples were defined as the ratio of each specific primer to GAPDH expression.

Statistical analysis

SPSS for Windows (version 15.0; SPSS, Chicago, IL, USA) was used to perform the data analysis. The data were subjected to the Shapiro–Wilk test to characterize normality. Because the samples did not present a normal distribution, the Wilcoxon test was used to determine significant differences ($P < 0.05$).

Table 1: Primer sequences

Gene	Sense and antisense	Mt (°C)	bp
GAPDH	5'-GCA CCA CCA ACT GCT TAG CA-3'	80	96
	5'-TGG CAG TGA TGG CAT GGA GGA-3'		
IFN- γ	5'-GAA CTG TCG CCA GCA GCT AAA-3'	80	95
	5'-TGC AGG CAG GAC AAC CAT TA-3'		
IL-1β	5'-TGG CAG AAA GGG AAC AGA A- 3'	73	59
	5'-ACA ACA GGA AAG TCC AGG CTA- 3'		
TNF-α	5'-TTC TGG CTC AAA AAG AGA ATT G- 3'	76	73
	5'-TGG TGG TCT TGT TGC TTA AGG- 3'		
IL-10	5'-GGT TGC CAA GCC TTG TCT GA- 3'	81	107
	5'-TCC CCC AGG GAG TTC ACAT- 3'		
CCL2/MCP-1	5'-AAG ACC ATT GTG GCC AAG GA- 3'	80	93
	5'-CGG AGT TTG GGT TTG CTT GT- 3'		
CXCR4	5'-GAA CTG TCG CCA GCA GCT AAA-3'	80	71
	5'-TGC AGG CAG GAC AAC CAT TA-3'		
CCL4	5'- TCT CCT CAT GCT AGT AGC TGC CTT- 3'	78	101
	5'- GCT TCC TCG CAG TGT AAG AAA AG-3'		

Mt: melting temperature; bp: base pairs of amplicon size.

Results

Levels of mRNA expression were determined by real-time PCR and were quantified by comparison with the internal control gene, GAPDH. The results revealed significant increases in the expression of TNF-α and IFN-γ mRNA in

teeth with restrained bacterial loads (day 7) compared to the first collection (day 0) in both pre-HSCT (bone marrow group) and healthy individuals (control group). However, the mRNA expression levels of TNF-α and IFN-γ were significantly higher in the healthy/control group than in the bone marrow group obtained on day 7. Similarly, the mRNA expression levels of IL-1-β and IL-10 in the bone marrow group increased in samples from day 7 ($P < 0.05$) (FIGURE 1).

Chemokine receptor (CXCR4) mRNA expression increased in samples from day 7 ($P < 0.05$) in the healthy/control group. The expression of CCL-4 was not significantly different between the groups. CCL-2/MCP-1 mRNA expression was not detected in either the first or second samples in bone marrow individuals but was present in periapical samples obtained from healthy/control individuals and in a positive primer amplification control, which was assayed in duplicate in the same well plate (FIGURE 2).

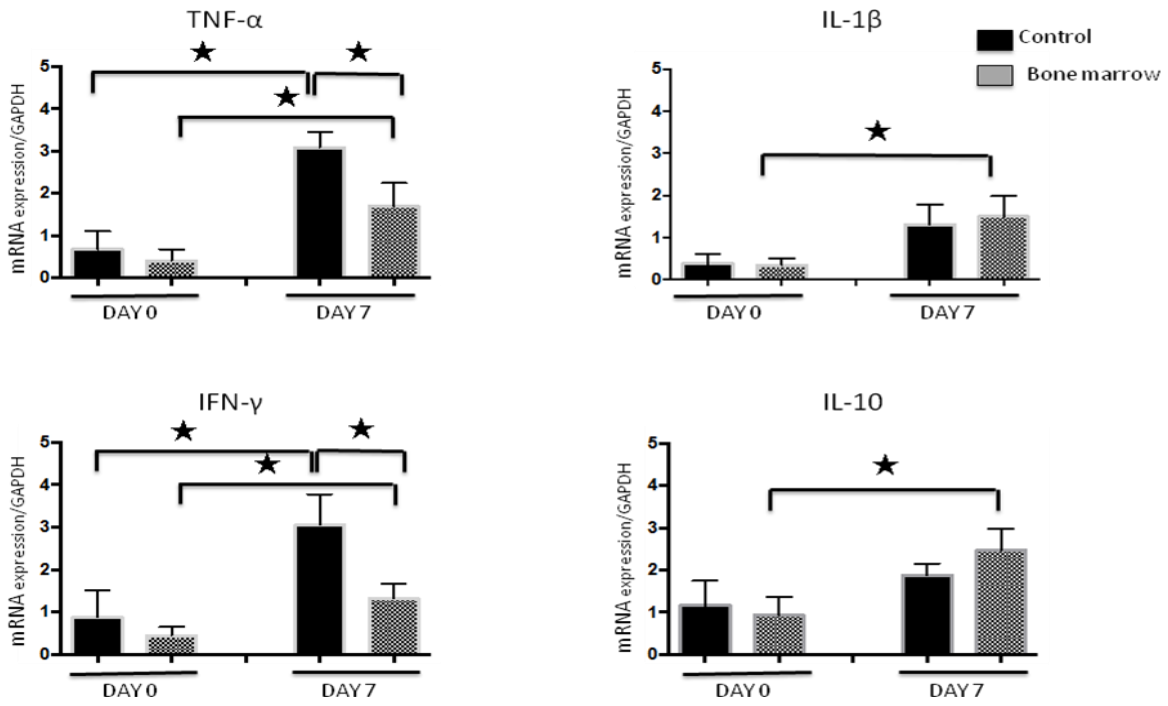

Figure 1: Expression of TNF-α, IFN-γ, IL-1β and IL-10 in periradicular tissue obtained from healthy/control individuals and pre-HSCT patients with root canal infections. Expression levels were determined by real-time PCR and were quantified by comparison with an internal control (GAPDH). Bars represent the mean values of samples recovered from 10 healthy individuals and 10 pre-HSCT individuals, and lines represent the standard error of the mean. ★ Indicates $P<0.05$ according to the Wilcoxon test.

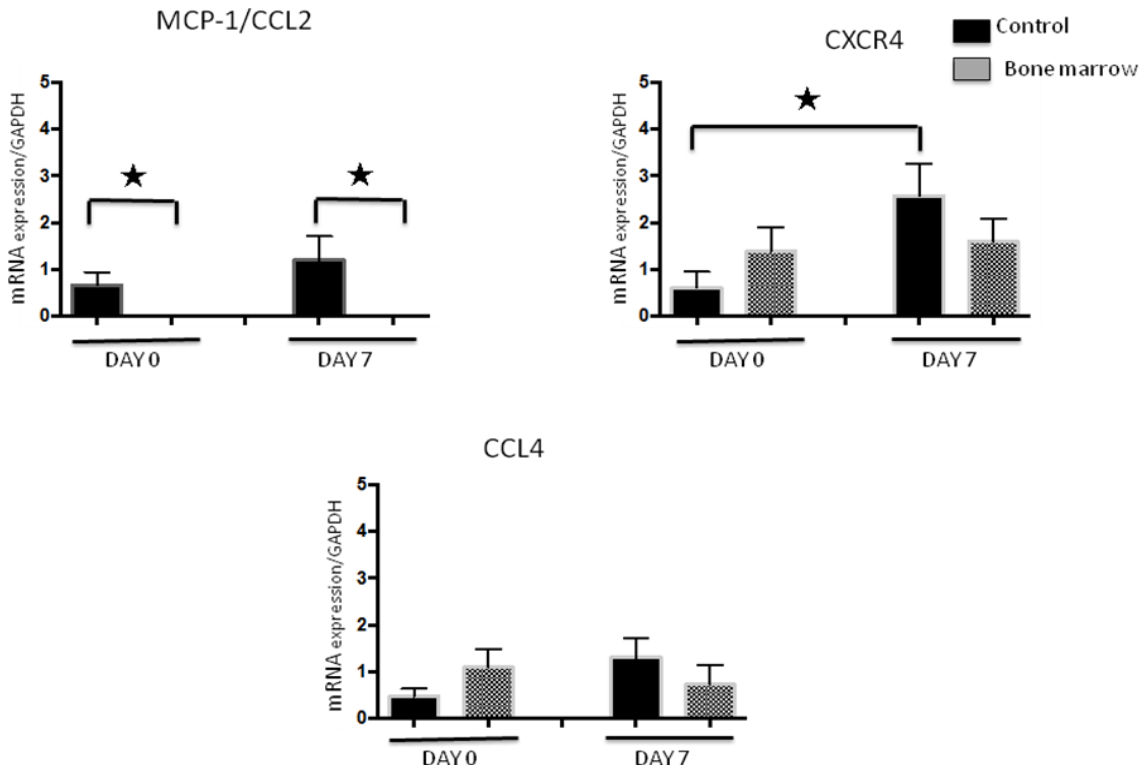

Figure 2: Expression of MCP1/CCL2, CXCR-4, CCL-4 in periradicular tissue obtained from healthy/control individuals and pre-HSCT patients with root canal infections. Expression levels were determined by real-time PCR and were quantified by comparison with an internal control (GAPDH). Bars represent the mean values of samples recovered from 10 healthy individuals and 10 pre-HSCT individuals, and lines represent the standard error of the mean. ★ Indicates $P<0.05$ according to the Wilcoxon test.

Discussion

Haematopoietic stem cell transplantation (HSCT) is widely used as a potentially curative treatment for patients with various haematological malignancies, bone marrow failure syndromes, and congenital immune deficiencies. HSCT makes patients more susceptible to infection because the oral cavity is a relevant source of pathogens that are likely to cause systemic disorders in such subjects (1). It is believed that infections of oral origin occur in approximately 80% of cases (15).

Pulpal and periradicular diseases usually result from the direct or indirect involvement of microorganisms that are present in the oral cavity (16, 17). The presence of microorganisms and their by-products inside infected root canals evoke the host immune response, which acts to avoid dissemination through the periapical foramen. Cleaning and shaping procedures strongly diminish the root canal bacterial load, and periapical healing starts soon afterward. This process involves overlapping phases of inflammation, proliferation, and remodelling. Each of these phases is characterized by dynamic interactions among components of the extracellular matrix, growth factors, and cells (4, 18). In this study, expression levels of inflammatory and regulatory cytokines and chemokines were investigated in the periapical lesions immediately after root canal cleaning procedures and 7 days later.

A type 1 immune response, which is characterized by the production of IFN-γ, TNF-α, and IL-1, is involved in the progression, bone destruction, and remodelling of periapical lesions (19). TNF-α is a proinflammatory cytokine that is released by activated monocytes, macrophages, and T lymphocytes, thus contributing to the immune response, growth regulation, differentiation, survival, and physiological function of a variety of cells and the production of other cytokines, inflammatory mediators, and enzymes (20, 21). This molecule is a potent inducer of bone resorption that stimulates the differentiation and activation of osteoclasts (22). IFN-γ is the main activator of macrophages, which subsequently produce cytokines and other mediators, thus playing a significant role in the development of periapical diseases (23). In this study, the mRNA expression levels of the proinflammatory cytokines TNF-α and IFN-γ increased on day 7 after cleaning and shaping procedures in a healthy/control group and

in a bone marrow group. Conversely, previous studies have reported a decrease in the expression levels of proinflammatory mediators after cleaning and shaping procedures (4, 10, 24). These contradictory results may be related to specific microbial challenge in each infected root canal and the patient's genetic make-up. A study that analysed different intracanal medications on Th1 and Th2 cytokine subtypes showed that proinflammatory cytokine expression levels in the periapical area were higher when higher bacterial counts in root canal infection were present (25). The results of our study revealed that the mRNA expression levels of TNF-α and IFN-γ were significantly higher in the healthy/control group than in the bone marrow group on day 7. Perhaps this finding was related to neutropenia, which has always been associated with patients undergoing HSCT (9). It has also been demonstrated that neutrophils play an active role in the development of bone loss associated with endodontic lesions (26).

Interleukins (IL), particularly IL-1β, are produced in periapical lesions by several types of cells, including macrophages, osteoclasts, PMNs, and fibroblasts (27). Among the local effects of IL-1, we wish to especially note lymphocyte stimulation, neutrophil potentiation, the production of proteases and prostaglandin activation, leukocyte adhesion enhancement, and bone formation inhibition (28). Moreover, using interleukin-1 receptor antagonists, it was shown that IL-1 is responsible for 60% of lesion development (29). It has previously been demonstrated that IL-1β is increased in periapical lesions (30, 31) and is decreased after endodontic treatment (24). Interestingly, in the bone marrow group studied here, the expression of IL-1β mRNA was significantly higher on day 7 (restrained bacterial load) than on day 0. This result suggests that on day

7, a type 1 immune response, characterized by the production of interferon-γ (IFN-γ), tumor necrosis factor-a (TNF-α), and interleukin-1β (IL-1β), which is involved in the progression, bone destruction, and remodeling of periapical lesions (5) yet remains. In the control group, the mRNA expression of IL-1β was similar between both times, as demonstrated elsewhere (4).

Chemokines direct the cell movements that are necessary for the initiation of T-cell immune responses and attract the appropriate effector cells to sites of inflammation, including the selective recruitment of Th1 and Th2 cells (7). Increased CCL2/MCP-1 expression has been associated with an increase in the recruitment of cells to inflammatory sites (32). Abundantly produced in chronic inflammation, MCP-1/CCL2 is associated with osteoclast chemotaxis and differentiation (33), mediates monocyte recruitment to bone inflammatory sites, and is involved in bone remodelling (34). In this study, CCL2 was found at similar levels in the healthy/control group on days 0 and 7 after root canal cleaning, as demonstrated previously (11). Interestingly, CCL-2/MCP-1 was not detected in the bone marrow group, which suggests that in those patients, the recruitment of appropriate effector cells to sites of inflammation was impaired. The consequence of this phenomenon for periapical immune responses is a matter of debate. An experimental study shows that mice lacking MCP-1 experience delayed wound healing and show delayed re-epithelialization and reduced capillary density (35).

CCL4 is a chemoattractant for natural killer cells and monocytes, and CXCR4 is an alpha-chemokine receptor that is potently chemotactic for lymphocytes. In the control group studied here, CCL4 mRNA expression did not change when the bacterial load was restrained after cleaning and shaping the

root canal. However, CXCR4 increased after root canal therapy in this group, as previously shown (24). It was recently demonstrated that CXCR4 is a natural ligand of ubiquitin (36), a protein that is highly conserved among eukaryotic cells and that acts as an anti-inflammatory immune modulator (37). This finding might suggest that the healing procedures that occur after root canal treatment may be modulated in healthy patients by this mechanism. Additionally, in the bone marrow group, no significant differences were observed in CXCR4/CCL4 expression between the two times analysed.

Immunosuppressive mechanisms mediated by IL-10 are responsible for healing processes and inflammatory/immune mechanism restriction. The importance of IL-10 for controlling the degree and duration of inflammatory reactions has been observed in several chronic inflammatory and autoimmune pathologies (12, 38, 39). No significant differences in IL-10 expression in the healthy/control group were observed between days 0 and 7, possibly due to cross-immune regulation promoted by the high expression of proinflammatory cytokines (TNF-α and lFN-γ) on day 7. This result reinforces the previous observation about the healing process in the control group: this was not modulated by IL-10 but was probably modulated by CXCR4. Conversely, in the bone marrow group, high mRNA IL-10 expression was observed on day 7 (after bacterial load restraint), suggesting that the immunoregulatory process occurs as the result of IL-10 interference.

The outcomes found in this study generally demonstrated similar cytokine and chemokine mRNA expression in healthy individuals and in those undergoing HSTC. However, it is worth noting the role of the total absence of mRNA MCP-1/CCL2 expression in those individuals undergoing HSCT and its

consequences for the periapical immune response. Moreover, the immune regulatory process seems to be modulated differently in both groups; in healthy individuals, the process is related to CXCR4, while in individuals undergoing HSTC, it is related to IL-10. Further immunologic and prospective studies are necessary to better understand the interesting results observed herein and their consequences for the periapical immune responses that occur in patients undergoing HSCT.

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References

1. Haverman TM, Raber-Durlacher JE, Rademacher WM, Vokurka S, Epstein JB, Huisman C, et al. Oral complications in hematopoietic stem cell recipients: the role of inflammation. Mediators Inflamm. 2014;2014:378281.

2. Forman SJ, Blume KG, Thomas ED. Hematopoietic cell transplantation: Blackwell Science; 1999.

3. de Brito LC, Teles FR, Teles RP, Totola AH, Vieira LQ, Sobrinho AP. T-lymphocyte and cytokine expression in human inflammatory periapical lesions. J Endod. 2012;38(4):481-5.

4. Bambirra W, Jr., Maciel KF, Thebit MM, de Brito LC, Vieira LQ, Sobrinho AP. Assessment of Apical Expression of Alpha-2 Integrin, Heat Shock Protein, and Proinflammatory and Immunoregulatory Cytokines in Response to Endodontic Infection. J Endod. 2015;41(7):1085-90.

5. Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol. 2011;3.

6. Romagnani S. T-cell subsets (Th1 versus Th2). Ann Allergy Asthma Immunol. 2000;85(1):9-18; quiz , 21.

7. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol. 2000;18:593-620.

8. Bryant VL, Slade CA. Chemokines, their receptors and human disease: the good, the bad and the itchy. Immunol Cell Biol. 2015;93(4):364-71.

9. Sviland L, Pearson AD, Green MA, Baker BD, Eastham EJ, Reid MM, et al. Immunopathology of early graft-versus-host disease--a prospective study of

skin, rectum, and peripheral blood in allogeneic and autologous bone marrow transplant recipients. Transplantation. 1991;52(6):1029-36.

10. Tavares WL, de Brito LC, Henriques LC, Teles FR, Teles RP, Vieira LQ, et al. Effects of calcium hydroxide on cytokine expression in endodontic infections. J Endod. 2012;38(10):1368-71.

11. Tavares WL, de Brito LC, Henriques LC, Oliveira RR, Maciel KF, Vieira LQ, et al. The impact of chlorhexidine-based endodontic treatment on periapical cytokine expression in teeth. J Endod. 2013;39(7):889-92.

12. Henriques LC, de Brito LC, Tavares WL, Vieira LQ, Ribeiro Sobrinho AP. Cytokine analysis in lesions refractory to endodontic treatment. J Endod. 2011;37(12):1659-62.

13. Barbosa Silva MJ, Vieira LQ, Sobrinho AP. The effects of mineral trioxide aggregates on cytokine production by mouse pulp tissue. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(5):e70-6.

14. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.

15. Greinix HT, Nachbaur D, Krieger O, Eibl M, Knobl P, Kalhs P, et al. Factors affecting long-term outcome after allogeneic haematopoietic stem cell transplantation for acute myelogenous leukaemia: a retrospective study of 172 adult patients reported to the Austrian Stem Cell Transplantation Registry. Br J Haematol. 2002;117(4):914-23.

16. Fabricius L, Dahlen G, Holm SE, Moller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res. 1982;90(3):200-6.

17. Sundqvist GK, Eckerbom MI, Larsson AP, Sjogren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infect Immun. 1979;25(2):685-93.

18. Bakhshayesh M, Soleimani M, Mehdizadeh M, Katebi M. Effects of TGF-beta and b-FGF on the potential of peripheral blood-borne stem cells and bone marrow-derived stem cells in wound healing in a murine model. Inflammation. 2012;35(1):138-42.

19. Takeichi O, Saito I, Tsurumachi T, Moro I, Saito T. Expression of inflammatory cytokine genes in vivo by human alveolar bone-derived polymorphonuclear leukocytes isolated from chronically inflamed sites of bone resorption. Calcif Tissue Int. 1996;58(4):244-8.

20. Bradley JR. TNF-mediated inflammatory disease. J Pathol. 2008;214(2):149-60.

21. Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling. Cell Death Differ. 2003;10(1):45-65.

22. Kwan Tat S, Padrines M, Theoleyre S, Heymann D, Fortun Y. IL-6, RANKL, TNF-alpha/IL-1: interrelations in bone resorption pathophysiology. Cytokine Growth Factor Rev. 2004;15(1):49-60.

23. Colic M, Lukic A, Vucevic D, Milosavljevic P, Majstorovic I, Marjanovic M, et al. Correlation between phenotypic characteristics of mononuclear cells isolated from human periapical lesions and their in vitro production of Th1 and Th2 cytokines. Arch Oral Biol. 2006;51(12):1120-30.

24. de Brito LC, Teles FR, Teles RP, Nogueira PM, Vieira LQ, Ribeiro Sobrinho AP. Immunological profile of periapical endodontic infections from HIV- and HIV+ patients. Int Endod J. 2015;48(6):533-41.

25. Martinho FC, Nascimento GG, Leite FR, Gomes AP, Freitas LF, Camoes IC. Clinical influence of different intracanal medications on Th1-type and Th2-type cytokine responses in apical periodontitis. J Endod. 2015;41(2):169-75.

26. Yamasaki M, Kumazawa M, Kohsaka T, Nakamura H. Effect of methotrexate-induced neutropenia on rat periapical lesion. Oral Surg Oral Med Oral Pathol. 1994;77(6):655-61.

27. Fouad AF. IL-1 alpha and TNF-alpha expression in early periapical lesions of normal and immunodeficient mice. J Dent Res. 1997;76(9):1548-54.

28. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15(6):348-81.

29. Stashenko P, Wang CY, Tani-Ishii N, Yu SM. Pathogenesis of induced rat periapical lesions. Oral Surg Oral Med Oral Pathol. 1994;78(4):494-502.

30. Ferreira SB, Tavares WL, Rosa MA, Brito LC, Vieira LQ, Martelli HJ, et al. Sickle cell anemia in Brazil: personal, medical and endodontic patterns. Braz Oral Res. 2016;30(1).

31. Morsani JM, Aminoshariae A, Han YW, Montagnese TA, Mickel A. Genetic predisposition to persistent apical periodontitis. J Endod. 2011;37(4):455-9.

32. Silva TA, Garlet GP, Lara VS, Martins W, Jr., Silva JS, Cunha FQ. Differential expression of chemokines and chemokine receptors in inflammatory periapical diseases. Oral Microbiol Immunol. 2005;20(5):310-6.

33. Kim MS, Day CJ, Selinger CI, Magno CL, Stephens SR, Morrison NA. MCP-1-induced human osteoclast-like cells are tartrate-resistant acid phosphatase, NFATc1, and calcitonin receptor-positive but require receptor

activator of NFkappaB ligand for bone resorption. J Biol Chem. 2006;281(2):1274-85.

34. Graves DT, Jiang Y, Valente AJ. Regulated expression of MCP-1 by osteoblastic cells in vitro and in vivo. Histol Histopathol. 1999;14(4):1347-54.

35. Low QE, Drugea IA, Duffner LA, Quinn DG, Cook DN, Rollins BJ, et al. Wound healing in MIP-1alpha(-/-) and MCP-1(-/-) mice. Am J Pathol. 2001;159(2):457-63.

36. Saini V, Marchese A, Majetschak M. CXC chemokine receptor 4 is a cell surface receptor for extracellular ubiquitin. J Biol Chem. 2010;285(20):15566-76.

37. Majetschak M. Extracellular ubiquitin: immune modulator and endogenous opponent of damage-associated molecular pattern molecules. J Leukoc Biol. 2011;89(2):205-19.

38. Bazzoni F, Tamassia N, Rossato M, Cassatella MA. Understanding the molecular mechanisms of the multifaceted IL-10-mediated anti-inflammatory response: lessons from neutrophils. Eur J Immunol. 2010;40(9):2360-8.

39. Sasaki H, Okamatsu Y, Kawai T, Kent R, Taubman M, Stashenko P. The interleukin-10 knockout mouse is highly susceptible to Porphyromonas gingivalis-induced alveolar bone loss. J Periodontal Res. 2004;39(6):432-41.

Artigo 4: Immunological profile of teeth with inflammatory periapical disease from chronic liver disease patients

Immunological profile of teeth with inflammatory periapical disease from chronic liver disease patients

Abstract

Patients with chronic liver diseases (CLD) demonstrate an increased susceptibility to infection due to a number contributory and predisposing factors. The aim of this study was to evaluate the mRNA expression levels of the cytokines interferon-γ, tumour necrosis factor-α, interleukin (IL)-1β, IL-10, IL-6, VEGF, and AGT and the chemokine CCL2/MCP-1 in periapical interstitial fluid from root canal infections before and after the reduction of the bacterial load using a cleaning procedure.The case group included 11 patients with chronic liver disease, and the control group included 11 healthy patients. Clinical samples were taken from teeth with pulp necrosis. After cleaning and drying, 3 paper points were introduced into the root canal and passed through the root apex (2 mm) into the periapical tissues for 1 min. The samples were collected immediately after root canal cleaning and 7 days later to characterize those gene expression levels using real-time PCR. In the control group, significantly increased expression of the proinflammatory cytokines IFN-γ and TNF-α was observed in teeth with restrained bacterial loads (day 7). Similarly, increased TNF-α expression was observed on day 7 in the liver group. The IL-β mRNA levels were higher in the liver group than in the control group at the first collection (day 0). Increased AGT mRNA levels in teeth with restrained bacterial loads compared with those from the first collection in control individuals.CLD patients exhibited sufficient immunologic ability showing relatively similar expression levels of cytokines, chemokines and angiogenic factors in periapical samples compared with the responses from non-CLD patients. The outcomes of this study suggest that liver impairment did not compromise the periapical immune response.

Keywords: liver transplant, periapical lesion, endodontics, cytokines, angiogenic factors.

Introduction

Endodontic lesions typically develop from exposure of the pulpal tissue to oral bacteria as a result of deficiencies in tooth integrity. This may result from carious lesions that dissolve the mineralized dental tissue, fractures of the tooth structure, or iatrogenic or other circumstances that allow bacteria to penetrate into the pulpal tissues. In most cases, these events lead to SCR infection, which causes the development of perirradicular inflammation. As expected, the host response plays a critical and protective role in lesions of endodontic origin (1).
The host immune response is complex and involves the recruitment of inflammatory cells and the production of cytokines and chemokines. The antigen-presenting cells, especially dendritic cells and macrophages, are at least responsible for the polarization of 4 different Th subsets (2). The inflammatory response is related to the Th1 subset, which produces cytokines such as interferon gamma (IFN-g), tumor necrosis factor alpha (TNF-a), and interleukin (IL-1). These molecules are involved in periapical lesion progression, bone destruction, and remodelling (3). Conversely, the healing process is related to the Th2 subset. The Th17 subset may play a role in exacerbating inflammation by stimulating the secretion of proinflammatory mediators, such as IL-8, TNF-a, and IL-6 (4). Regulatory T (Treg) cells maintain normal homeostasis and reduce Th1, Th2, and Th17 over activity. IL-10 exhibits strong anti-inflammatory properties and is produced by Th1, Th17, Th2, and Treg cells (2).

Patients with chronic liver disease (CLD), particularly those with chronic viral hepatitis C or those in which liver impairment is associated with alcohol abuse, tend to present with deficient oral hygiene (5, 6). These diseases may be associated with lifestyles and behaviours that contribute to dental neglect and untreated dental disease (7). Moreover, a number of features associated with advanced liver disease can diminish saliva production, which in turn promotes the deposition and retention of dental plaque and increases patient vulnerability to caries (5). Dental caries may provide points of entry for bacteria into the pulpal space, which can evolve to pulpal necrosis. If appropriate endodontic treatment is not provided, the infected root canal system become an

indissoluble source of bacteria that has the potential to induce apical periodontitis (8).

It has been estimated that 60 to 80% of liver transplant recipients develop an infection, and infections can compromise the survival of any organ transplant recipient. Patients with CLD considered for liver transplantation should be examined for the presence of dental infection, which should be treated before transplantation, since they may be a source of bacterial infection if immunosuppressive treatment is initiated (9). Systemic conditions and disorders can modulate factors that affect oral infection progression rather than acting as causative aetiologic factors (10, 11). A recent study reported that CLD patients are at 4-fold increased risk of mortality after infection due to their decreased immune system function and present with increased levels of endotoxins, nitric oxide and cytokines, such as tumor necrosis factor-α and interleukin-6 (12). Castellanos-Cosano *et al* (13) reported that teeth in patients with liver transplants were more associated with endodontic pathosis compared with the control group. However, no study has analysed the periapical immune responses to root canal infections in patients with CLD, who exhibited impaired immunologic responses.

The initiation of an inflammatory cascade in lesions of endodontic origin includes the complex interplay of multiple cell types and involves the activation of endothelial cells, PMNs, macrophages, lymphocytes, and osteoclasts, leading to rapid bone destruction. . The aim of this study was to quantitatively assay the expression of IFN-γ, TNF-α, IL-1β, IL-6, IL-10, CCL-2/MCP-1, VEGF and AGT in samples collected from interstitial fluid adjacent to root canal infections in healthy/control and pre-Liver transplant group individuals. Seven days later, these same parameters were assayed following root canal cleaning procedures, which significantly reduced root canal bacterial load and after which the healing process had begun, as demonstrate elsewhere (14, 15).

Materials and Methods

Human subjects

The subjects included 11 healthy patients and 11 patients with CLD who were selected from 120 males and females (children and adults) enrolled in the dental care programme for transplanted patients of the School of Dentistry, Federal University of Minas Gerais (Faculdade de Odontologia da Universidade Federal de Minas Gerais, FO-UFMG) from March 2011 through March 2016. All patients updated their medical records at the first appointment. The patients were subjected to a conditioning dental regimen based on a specific protocol applied at the FO-UFMG. The conditioning dental regimen consisted of thorough clinical and radiographic evaluations. Intraoral periapical and bite-wing X-rays associated with panoramic radiograph were obtained. The selected patients had teeth with pulp necrosis and apical periodontitis and were between 26 and 65 years of age. All participants signed the Free Agreement Formulary. This study was approved by the Ethics Committee of the Universidade Federal de Minas Gerais (CAAE: 54829414.7.0000.5149/ruling:1.569.493).

Sample collection

Clinical samples were taken from teeth with pulp necrosis, which was diagnosed based on clinical and radiographic analyses and pulp sensibility testing. The teeth did not present acute periapical symptoms at the time of appointment. The sampling procedures were performed as previously described (16, 17). Each tooth was isolated, and the cleaning and shaping of the root canals were completed using ProTaper universal NiTi files (Dentsply Maillefer, Ballaigues, Switzerland) with 2.5% sodium hypochlorite. The samples were collected immediately after root canal cleaning for cytokine/chemokine expression profiling. After cleaning and drying, three paper points (#20) were introduced into the root canal and passed passively through the root apex (2 mm) into the periapical tissues for 1 min. The paper points were cut 4 mm from the tip and placed in a microcentrifuge tube for storage at -70 °C. In this procedure, RNA was extracted from the periapical interstitial fluid. No endodontic dressing was inserted into the root canals. The coronal access

cavities of the teeth were restored with a eugenol-based cement. Seven days later (day 7), the teeth were opened, and the periapical interstitial fluid was again sampled to characterize the cytokine/chemokine profiles in those teeth with restrained root canal bacterial loads, as demonstrated by others (18). Single and multiple root teeth were included in this study. In teeth with multiple canals, the first (day 0) and second (day 7) samples were collected from the same canal. At this time, no teeth had clinical signs or symptoms, and the root canals were filled using the lateral compaction technique.

Sample preparation

Total RNA was extracted from each sample using the TRIzol reagent (GIBCO/BRL Laboratories, Grand Island, NY) as described elsewhere (16, 17, 19).The RNA was then stored at -70 °C.

Real-time polymerase chain reaction

Complementary DNA was synthesized using 1 µg of RNA and the reverse transcription reaction as described previously (20). Primer sequences were designed using the PRIMER EXPRESS software (Applied Biosystems, Foster City, CA, USA) based on the nucleotide sequences available in the GenBank database. A real-time PCR assay was also performed using the PRIMER EXPRESS software (Applied Biosystems). The primer sequences used for the quantitative PCR analysis of IFN-γ, TNF-α, IL-1β, IL-10, Il-6, VEGF, AGT and MCP-1 mRNA expression are shown in Table 1. PCR was performed under standard conditions as follows: a holding stage at 95 °C (10 min); a cycling stage of 40 cycles at 95 °C (15 s) followed by 60 °C (1 min); and a melting curve stage at 95 °C (15 s), 60 °C (1 min) and 95 °C (15 s). A SYBR-Green detection system (Applied Biosystems) was used to assay primer amplification. Following amplification, melting curve analysis was performed to determine the specificity of the amplified products. The melting curve was obtained from 60 °C to 95 °C, with continuous fluorescence measurements taken at every 1% increase in temperature. PCR products with melting temperatures divergent from those established for standard DNA were considered as false-positives; a null fluorescence value was noted in such

cases. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for normalization and was analysed with each set of reactions. All samples were run in duplicate. Each reaction was performed in a 25 µL volume containing 1 µg of cDNA. The Sequence Detection System (SDS) Software version 2.4.1 (Applied Biosystems) was used to analyse data after amplification. The results were obtained as threshold cycle (Ct) values, and the expression levels were calculated using the comparative $2^{-\Delta\Delta CT}$ method (21). The values were calculated as the mean value of the duplicates for each patient, and the mRNA expression levels in all samples were defined as the ratio of each specific primer to that of GAPDH.

Table 1. Primer sequences, Melting Temperature (Mt), and Amplicon Sizes for Each Target Cytokine, Chemokine or Angiogenic Factors

Gene	Sense and antisense	Mt (°C)	bp
GAPDH	5'-GCA CCA CCA ACT GCT TAG CA-3'	80	96
	5'-TGG CAG TGA TGG CAT GGA GGA-3'		
IFN- γ	5'-GAA CTG TCG CCA GCA GCT AAA-3'	80	95
	5'-TGC AGG CAG GAC AAC CAT TA-3'		
IL-1β	5'-TGG CAG AAA GGG AAC AGA A- 3'	73	59
	5'-ACA ACA GGA AAG TCC AGG CTA- 3'		
TNF-α	5'-TTC TGG CTC AAA AAG AGA ATT G- 3'	76	73
	5'-TGG TGG TCT TGT TGC TTA AGG- 3'		
IL-10	5'-GGT TGC CAA GCC TTG TCT GA- 3'	81	107
	5'-TCC CCC AGG GAG TTC ACAT- 3'		
CCL2/MCP-1	5'-AAG ACC ATT GTG GCC AAG GA- 3'	80	93
	5'-CGG AGT TTG GGT TTG CTT GT- 3'		
IL-6	5'- GGA GAC TTG CCT GGT GAA- 3'	80	76
	5'- CTG GCT TGT TCC TCA CTA CTC-3'		
AGT	5'-ACA GTT TGG CAA TTG GAA GCA-3'	65	152
	5'-CAC CCA GAT GAC TCC AAG ATC AG3'		
VEGF	5′-ATC TGC ATG GTG ATG TTG GA-3′	71	214
	5′-GGG CAG AAT CAT CAC GAA GT-3′		

Statistical analysis

SPSS for Windows (version 15.0; SPSS, Chicago, IL, USA) was used to perform data analysis. The data were subjected to the Shapiro–Wilk test to characterize normality. Because the samples did not present a normal distribution, the Wilcoxon test was used to determine significant differences ($p < 0.05$).

Results

The levels of mRNA expression were determined by real-time PCR and quantified by comparison with the internal control gene GAPDH. Significantly increased expression of the proinflammatory cytokines IFN-γ and TNF-α was observed in teeth with restrained bacterial loads (day 7) in the control group (FIG 1). In the liver group, similarly increased expression of TNF-α in teeth with restrained bacterial loads was observed (day 7), however, the TNF-α and IFN-γ mRNA levels were significantly higher in the healthy/control group than in the liver group on day 7(FIG 1). When comparing the expression levels of the cytokines IL-1β, IL-10 and, IL-6 and the chemokines MCP-1/CCL-2 and VEGF between the first collection (day 0) and second collection (day 7), no significant differences were observed over time in either group ($p>.05$). However, the IL-β mRNA levels were higher in the liver group than in the healthy/control group at the first collection (day 0) (FIG 1). Assessment revealed a significant increase in AGT mRNA levels in teeth with restrained bacterial load (day 7) compared with the first collection in control individuals (FIG 2).

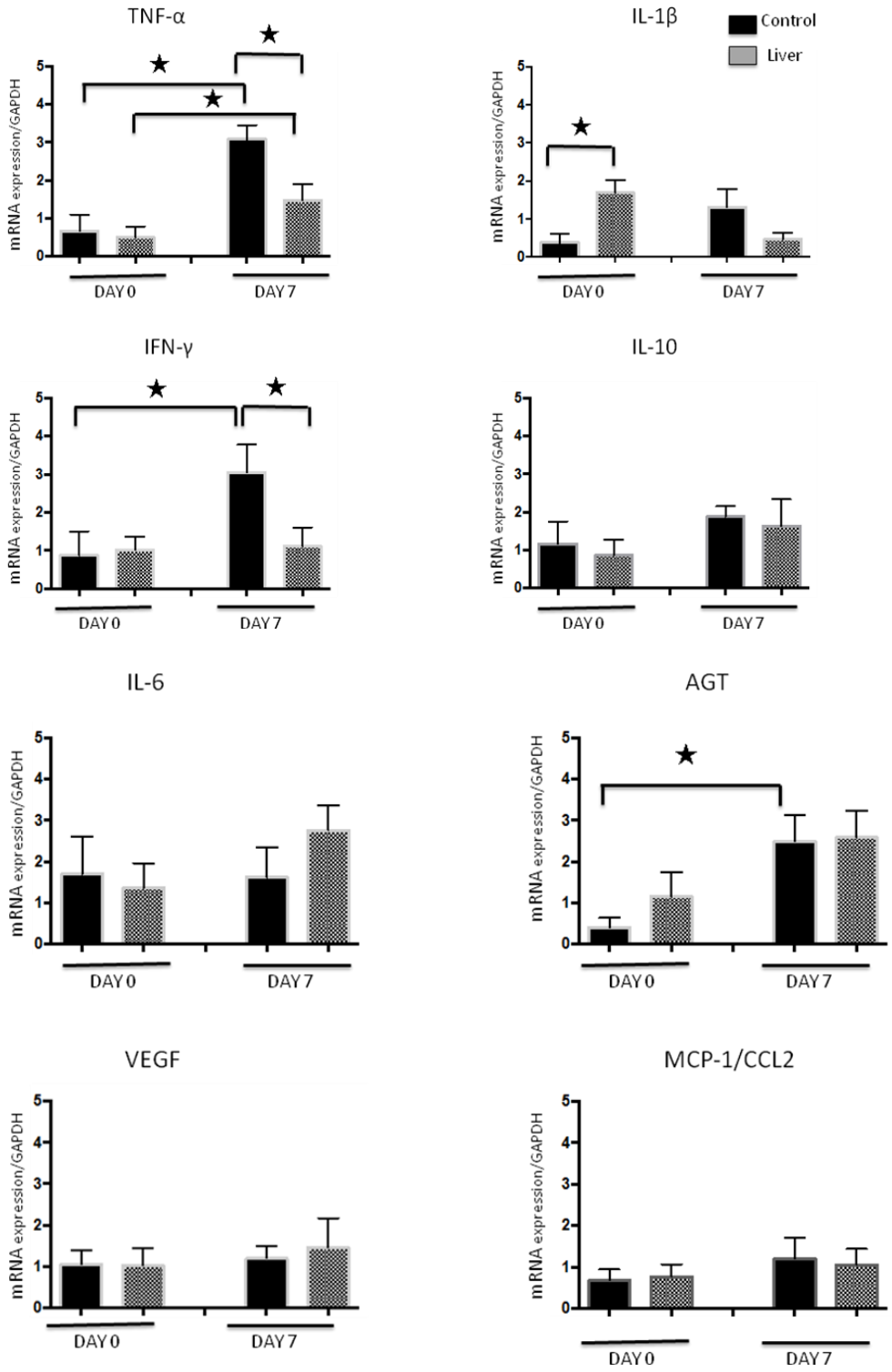

Figure 1: Expression of TNF-α, IFN-γ, IL-1β and IL-10,IL-6, AGT,VEGF and MCP-1/CCL-2 in perirradicular tissue from health/control and liver transplant patients with root canal infections. Levels of expression were determined by real-time PCR and quantified by comparison with the internal control (GAPDH). Bars represent the mean values of sample recovered from 11 health individuals and 11 pre-liver transplant individuals and lines represent the standard error of the mean. ★ Indicates $P<0.05$ by Wilcoxon test.

Discussion

Apical periodontitis is one of the most common inflammatory diseases in the oral cavity and is caused by bacterial infection of the root canal (22). This disease represents a host response to continuous antigenic stimulation and involves recruitment of inflammatory cells, generation of cytokines, elaboration of lytic enzymes, and activation of osteoclasts, which leads to periapical bone resorption (23). Pro-inflammatory status and impaired immune response associated with systemic diseases can affect the reparative response of the dental pulp and periapical healing (24). Marending et al. (25) reported that the integrity of the nonspecific immune system was a significant predictor for endodontic initial treatment and retreatment outcome. Ng et al. (26) also demonstrated the impact that an impaired nonspecific immune system had on the healing of periapical tissues.

Bacterial antigens induce proinflammatory cytokine production, including that of TNF-α, IFN-γ and IL-1β. Unexpectedly, in this study, greater expression of TNF-α and IFN-γ was observed after cleaning and shaping procedures in control/healthy group. Similarly, increased expression of TNF-α was observed in teeth with restrained bacterial loads (day 7) in the liver group. These contradictory results may be related to specific microbial challenge in each root canal infection, as well as, the individual genetic make-up. A study that analysed different intracanal medications on Th1 and Th2 cytokine subtypes showed that proinflammatory cytokine expression levels in the periapical area were higher when higher bacterial counts in root canal infection were present (27). Previous studies have already shown decreased expressions of proinflammatory mediators after cleaning and shaping procedures (17, 28). Moreover, in this study, TNF-α and IFN-γ mRNA expression levels were significantly higher in the healthy/control group than in the liver group on day 7. IFN-γ activates macrophages, reduces macrophage-suppressive activity, and plays a significant role in the development of periapical diseases (29).

IL-β mRNA levels were higher in the liver group than in the healthy/control group at the first collection (day 0). IL-1 has been identified as a central mediator of periapical and pulpal inflammation (23) that increases

leukocyte adhesion, stimulates lymphocytes, and, together with TNF-a, enhances periapical bone resorption while inhibiting bone formation (30). These results may be explained since the pattern and intensity of cytokine expression shows marked variation among individuals, depending on the source of stimulatory agent (31, 32).

It is well known that CLD patients respond to sepsis with a greater and longer-lasting increase in the circulating levels of IL-6 and TNF-α than do patients without cirrhosis (33, 34). Here, significant detectable levels of IL-6 were found in the liver and control group periapical samples, although no significant difference in IL-6 production was observed between the groups. IL-6 has been shown to play a protective role in periapical tissues. Endodontic lesions from IL-6-deficient animals are larger than those from control mice (35). Depletion of IL-6 also impairs immune cell recruitment to sites of infection and macrophage differentiation, which weaken host defences against infectious diseases (35).

MCP-1/CCL2 was similarly expressed in both groups and at each time point, as demonstrated elsewhere (19). MCP-1 is a chemokine that possess chemoattractant properties for a number of immune cells and mediates the recruitment of monocytes in several inflammatory models and diseases (36). MCP-1 is also associated with osteoclast chemotaxis and differentiation, probably through its interactions with the CCR2 receptor. Increased MCP-1 mRNA expression was observed in patients who presented with acute and refractory root canal treatment (16, 37).

Several studies have shown the role of IL-10 in periapical lesions, in periodontal diseases (28-30) and in refractory endodontic lesions (16). IL-10 is a cytokine that displays anti-inflammatory properties and plays a central role in reducing infection by limiting the immune response to pathogens and thereby preventing damage to the host (15). Using the same methodology of this study, other authors observed a higher level of IL-10 mRNA expression in periapical tissue after root canal cleaning in healthy individuals (15, 17, 28). However, opposite results were observed herein, as no differences in mRNA IL-10 expression were observed in either group between both time points. This may be explained by cross-immune regulation promoted by high expression of TNF-

α observed on day 7 as well as a later initiation of regulation of the periapical immune response. Perhaps, if we had investigated a later time point, we would have revealed a more clear-cut noninflammatory scenario.

Vascular components are essential to periapical inflammatory responses. Vascular endothelial growth factor (VEGF) is considered a key regulator of vascular permeability and one of the major inducers of angiogenesis (38). In this study, VEGF mRNA expression was observed in periapical lesions, although VEGF expression did not change when the bacterial load was restrained after cleaning and shaping root canal procedures in the control and liver groups. According to Leonardi et al. (39), the expression of VEGF in periapical lesions increases vascular permeability and is partially involved in the accumulation of inflammatory cells and cyst fluid. Accordingly, Nonaka et al. (40) concluded that the expression of VEGF is closely related to the intensity of the inflammatory infiltrate in periapical inflammatory lesions.

Assessment revealed significantly increased AGT mRNA expression in teeth with restrained bacterial loads (day 7) compared with samples from the first collection in control individuals. Angiopoietins are groups of proteins that participate in angiogenesis and lymphangiogenesis (41), in particular playing a critical role in the initiation and stabilization of angiogenesis (42). Different types of neuropeptides, including calcitonin gene-related peptide, neuropeptide Y, substance P, and vasoactive intestinal polypeptide, can modify the expression of the above-mentioned angiogenic growth factors secreted from pulp fibroblasts and may modulate angiogenesis in periapical lesions (42).

There is considerable complexity in examining the impact of cytokine signalling since cytokines have both destructive roles and important protective functions in antibacterial defence (43). Although previous studies have found impaired immunologic responses in individuals with CLD (44, 45), in this study, CLD patients presented comparable immunologic ability, showing relatively similar expression levels of cytokines, chemokines and angiogenic factors in periapical samples when compared with non-CLD patients. Taken together, the outcomes of this study suggest that liver impairment did not compromise the periapical immune response.

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References

1. Graves DT, Oates T, Garlet GP. Review of osteoimmunology and the host response in endodontic and periodontal lesions. J Oral Microbiol. 2011;3.
2. Fukada SY, Silva TA, Garlet GP, Rosa AL, da Silva JS, Cunha FQ. Factors involved in the T helper type 1 and type 2 cell commitment and osteoclast regulation in inflammatory apical diseases. Oral Microbiol Immunol. 2009;24(1):25-31.
3. Takeichi O, Saito I, Tsurumachi T, Moro I, Saito T. Expression of inflammatory cytokine genes in vivo by human alveolar bone-derived polymorphonuclear leukocytes isolated from chronically inflamed sites of bone resorption. Calcif Tissue Int. 1996;58(4):244-8.
4. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol. 1999;44(1):55-66.
5. Guggenheimer J, Eghtesad B, Close JM, Shay C, Fung JJ. Dental health status of liver transplant candidates. Liver Transpl. 2007;13(2):280-6.
6. Coates EA, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, et al. Hepatitis C infection and associated oral health problems. Aust Dent J. 2000;45(2):108-14.
7. DiMartini A, Dew MA, Javed L, Fitzgerald MG, Jain A, Day N. Pretransplant psychiatric and medical comorbidity of alcoholic liver disease patients who received liver transplant. Psychosomatics. 2004;45(6):517-23.
8. Sasaki H, Hirai K, Martins CM, Furusho H, Battaglino R, Hashimoto K. Interrelationship Between Periapical Lesion and Systemic Metabolic Disorders. Curr Pharm Des. 2016;22(15):2204-15.
9. Anand AC, Pardal PK, Sachdev VP. DENTAL CARIES AND PERIODONTAL DISORDERS IN CHRONIC LIVER DISEASE. Med J Armed Forces India. 2001;57(1):26-30.
10. Fouad AF, Burleson J. The effect of diabetes mellitus on endodontic treatment outcome: data from an electronic patient record. J Am Dent Assoc. 2003;134(1):43-51; quiz 117-8.

11. Joshipura KJ, Pitiphat W, Hung HC, Willett WC, Colditz GA, Douglass CW. Pulpal inflammation and incidence of coronary heart disease. J Endod. 2006;32(2):99-103.
12. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010;139(4):1246-56, 56.e1-5.
13. Castellanos-Cosano L, Machuca-Portillo G, Segura-Sampedro JJ, Torres-Lagares D, Lopez-Lopez J, Velasco-Ortega E, et al. Prevalence of apical periodontitis and frequency of root canal treatments in liver transplant candidates. Med Oral Patol Oral Cir Bucal. 2013;18(5):e773-9.
14. de Brito LC, Teles FR, Teles RP, Totola AH, Vieira LQ, Sobrinho AP. T-lymphocyte and cytokine expression in human inflammatory periapical lesions. J Endod. 2012;38(4):481-5.
15. Bambirra W, Jr., Maciel KF, Thebit MM, de Brito LC, Vieira LQ, Sobrinho AP. Assessment of Apical Expression of Alpha-2 Integrin, Heat Shock Protein, and Proinflammatory and Immunoregulatory Cytokines in Response to Endodontic Infection. J Endod. 2015;41(7):1085-90.
16. Henriques LC, de Brito LC, Tavares WL, Vieira LQ, Ribeiro Sobrinho AP. Cytokine analysis in lesions refractory to endodontic treatment. J Endod. 2011;37(12):1659-62.
17. Tavares WL, de Brito LC, Henriques LC, Teles FR, Teles RP, Vieira LQ, et al. Effects of calcium hydroxide on cytokine expression in endodontic infections. J Endod. 2012;38(10):1368-71.
18. Rodrigues RCV, Zandi H, Kristoffersen AK, Enersen M, Mdala I, Orstavik D, et al. Influence of the Apical Preparation Size and the Irrigant Type on Bacterial Reduction in Root Canal-treated Teeth with Apical Periodontitis. J Endod. 2017;43(7):1058-63.
19. Tavares WL, de Brito LC, Henriques LC, Oliveira RR, Maciel KF, Vieira LQ, et al. The impact of chlorhexidine-based endodontic treatment on periapical cytokine expression in teeth. J Endod. 2013;39(7):889-92.

20. Barbosa Silva MJ, Vieira LQ, Sobrinho AP. The effects of mineral trioxide aggregates on cytokine production by mouse pulp tissue. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;105(5):e70-6.
21. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.
22. Bletsa A, Virtej A, Berggreen E. Vascular endothelial growth factors and receptors are up-regulated during development of apical periodontitis. J Endod. 2012;38(5):628-35.
23. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med. 1998;9(4):498-521.
24. Segura-Egea JJ, Martin-Gonzalez J, Castellanos-Cosano L. Endodontic medicine: connections between apical periodontitis and systemic diseases. Int Endod J. 2015;48(10):933-51.
25. Marending M, Peters OA, Zehnder M. Factors affecting the outcome of orthograde root canal therapy in a general dentistry hospital practice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;99(1):119-24.
26. Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature -- Part 2. Influence of clinical factors. Int Endod J. 2008;41(1):6-31.
27. Martinho FC, Nascimento GG, Leite FR, Gomes AP, Freitas LF, Camoes IC. Clinical influence of different intracanal medications on Th1-type and Th2-type cytokine responses in apical periodontitis. J Endod. 2015;41(2):169-75.
28. de Brito LC, Teles FR, Teles RP, Nogueira PM, Vieira LQ, Ribeiro Sobrinho AP. Immunological profile of periapical endodontic infections from HIV- and HIV+ patients. Int Endod J. 2015;48(6):533-41.
29. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med. 2004;15(6):348-81.
30. Sasaki H, Balto K, Kawashima N, Eastcott J, Hoshino K, Akira S, et al. Gamma interferon (IFN-gamma) and IFN-gamma-inducing cytokines interleukin-12 (IL-12) and IL-18 do not augment infection-stimulated bone resorption in vivo. Clin Diagn Lab Immunol. 2004;11(1):106-10.
31. Morandini AC, Sipert CR, Gasparoto TH, Greghi SL, Passanezi E, Rezende ML, et al. Differential production of macrophage inflammatory protein-

1alpha, stromal-derived factor-1, and IL-6 by human cultured periodontal ligament and gingival fibroblasts challenged with lipopolysaccharide from P. gingivalis. J Periodontol. 2010;81(2):310-7.

32. Scheres N, Laine ML, de Vries TJ, Everts V, van Winkelhoff AJ. Gingival and periodontal ligament fibroblasts differ in their inflammatory response to viable Porphyromonas gingivalis. J Periodontal Res. 2010;45(2):262-70.

33. Byl B, Roucloux I, Crusiaux A, Dupont E, Deviere J. Tumor necrosis factor alpha and interleukin 6 plasma levels in infected cirrhotic patients. Gastroenterology. 1993;104(5):1492-7.

34. Zeni F, Tardy B, Vindimian M, Comtet C, Page Y, Cusey I, et al. High levels of tumor necrosis factor-alpha and interleukin-6 in the ascitic fluid of cirrhotic patients with spontaneous bacterial peritonitis. Clin Infect Dis. 1993;17(2):218-23.

35. Huang GT, Do M, Wingard M, Park JS, Chugal N. Effect of interleukin-6 deficiency on the formation of periapical lesions after pulp exposure in mice. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2001;92(1):83-8.

36. Strieter RM, Standiford TJ, Huffnagle GB, Colletti LM, Lukacs NW, Kunkel SL. "The good, the bad, and the ugly." The role of chemokines in models of human disease. J Immunol. 1996;156(10):3583-6.

37. Sette-Dias AC, Maciel KF, Abdo EN, Brito LC, Carvalho MA, Vieira LQ, et al. Cytokine Expression in Patients Hospitalized for Severe Odontogenic Infection in Brazil. J Endod. 2016;42(5):706-10.

38. Nagy JA, Benjamin L, Zeng H, Dvorak AM, Dvorak HF. Vascular permeability, vascular hyperpermeability and angiogenesis. Angiogenesis. 2008;11(2):109-19.

39. Leonardi R, Caltabiano M, Pagano M, Pezzuto V, Loreto C, Palestro G. Detection of vascular endothelial growth factor/ vascular permeability factor in periapical lesions. J Endod. 2003;29(3):180-3.

40. Nonaka CF, Maia AP, Nascimento GJ, de Almeida Freitas R, Batista de Souza L, Galvao HC. Immunoexpression of vascular endothelial growth factor in periapical granulomas, radicular cysts, and residual radicular cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106(6):896-902.

41. Thurston G. Role of Angiopoietins and Tie receptor tyrosine kinases in angiogenesis and lymphangiogenesis. Cell Tissue Res. 2003;314(1):61-8.
42. El Karim IA, Linden GJ, Irwin CR, Lundy FT. Neuropeptides regulate expression of angiogenic growth factors in human dental pulp fibroblasts. J Endod. 2009;35(6):829-33.
43. Graves DT, Chen CP, Douville C, Jiang Y. Interleukin-1 receptor signaling rather than that of tumor necrosis factor is critical in protecting the host from the severe consequences of a polymicrobe anaerobic infection. Infect Immun. 2000;68(8):4746-51.
44. Caly WR, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. J Hepatol. 1993;18(3):353-8.
45. Varchetta S, Mele D, Mantovani S, Oliviero B, Cremonesi E, Ludovisi S, et al. Impaired intrahepatic natural killer cell cytotoxic function in chronic hepatitis C virus infection. Hepatology. 2012;56(3):841-9.

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

Pode-se observar que: a) As principais doenças que levaram a necessidade ou ao TCTH foram: as leucemias e o Mieloma Múltiplo; b) as principais doenças que levaram a necessidade ou ao TF foram a cirrose hepática e as hepatites virais; c) os indivíduos do sexo masculino representaram a maioria em ambos os grupos de estudo; d) foi constatada a necessidade de tratamento endodôntico, nos pacientes pré e pós TCTH, de 24.3% e 24.7% respectivamente; e) a necessidade de tratamento endodôntico observada nos pacientes pré TF foi de 20.7%; f) a maioria dos pacientes fazem uso de algum tipo de medicamento; g) os antibióticos representam a medicação mais utilizada no grupo TCTH enquanto os diuréticos representam a medicação mais utilizada no grupo TF; h) Indivíduos pré TCTH e pré TF, apresentam baixa contagem de células, principalmente plaquetas e hemoglobina; i) a expressão gênica das citocinas e quimiocinas demonstra que os pacientes pré TCTH possuem uma resposta similar a observada no grupo controle; j) a quimiocina MCP-1 não foi detectada no grupo pré TCTH; e i) a expressão gênica das citocinas, quimiocinas e dos fatores angiogênicos foi similar a observada no grupo controle.

Os resultados nos permite concluir que: a) a porcentagem de indivíduos com necessidade de tratamento endodôntico nos grupos de estudo é elevada; b) o uso de antibiótico reportado pela maioria dos pacientes pós TCTH ilustra a necessidade do controle de infecções na fase pós TCTH desses pacientes; c) a maioria pré TCTH e pré TF apresentam baixa contagem de células, o que pode estar relacionado com o comprometimento imune desses pacientes; d) a expressão gênica das citocinas e quimiocinas demonstra que os pacientes pré TCTH apresentam uma resposta pró inflamatória e anti-inflamatória eficaz; e e) a expressão gênica das citocinas, quimiocinas e dos fatores angiogênicos sugere que o comprometimento hepático não compromete a resposta imune periapical.

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REFERÊNCIAS (INTRODUÇÃO)

Referências

1. Fabricius L, Dahlen G, Holm SE, Moller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res. 1982;90(3):200-6.

2. Moller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. Odontol Tidskr. 1966;74(5):Suppl:1-380.

3. Sundqvist GK, Eckerbom MI, Larsson AP, Sjogren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. Infect Immun. 1979;25(2):685-93.

4. Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM. Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. J Immunol. 1987;138(5):1464-8.

5. Fukada SY, Silva TA, Garlet GP, Rosa AL, da Silva JS, Cunha FQ. Factors involved in the T helper type 1 and type 2 cell commitment and osteoclast regulation in inflammatory apical diseases. Oral Microbiol Immunol. 2009;24(1):25-31.

6. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med. 1998;9(4):498-521.

7. Silva TA, Garlet GP, Lara VS, Martins W, Jr., Silva JS, Cunha FQ. Differential expression of chemokines and chemokine receptors in inflammatory periapical diseases. Oral Microbiol Immunol. 2005;20(5):310-6.

8. Colic M, Gazivoda D, Vucevic D, Vasilijic S, Rudolf R, Lukic A. Proinflammatory and immunoregulatory mechanisms in periapical lesions. Mol Immunol. 2009;47(1):101-13.

9. Marton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol. 2000;15(3):139-50.

10. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity. 2008;28(4):445-53.

11. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol. 1999;44(1):55-66.

12. Teixeira-Salum TB, Rodrigues DB, Gervasio AM, Souza CJ, Rodrigues V, Jr., Loyola AM. Distinct Th1, Th2 and Treg cytokines balance in chronic periapical granulomas and radicular cysts. J Oral Pathol Med. 2010;39(3):250-6.

13. Yu JJ, Gaffen SL. Interleukin-17: a novel inflammatory cytokine that bridges innate and adaptive immunity. Front Biosci. 2008;13:170-7.

14. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol. 2000;18:593-620.

15. Kim MS, Day CJ, Selinger CI, Magno CL, Stephens SR, Morrison NA. MCP-1-induced human osteoclast-like cells are tartrate-resistant acid phosphatase, NFATc1, and calcitonin receptor-positive but require receptor activator of NFkappaB ligand for bone resorption. J Biol Chem. 2006;281(2):1274-85.

16. Bletsa A, Virtej A, Berggreen E. Vascular endothelial growth factors and receptors are up-regulated during development of apical periodontitis. J Endod. 2012;38(5):628-35.

17. Ferrara N. The role of VEGF in the regulation of physiological and pathological angiogenesis. Exs. 2005(94):209-31.

18. Nagy JA, Benjamin L, Zeng H, Dvorak AM, Dvorak HF. Vascular permeability, vascular hyperpermeability and angiogenesis. Angiogenesis. 2008;11(2):109-19.

19. Virtej A, Loes SS, Berggreen E, Bletsa A. Localization and signaling patterns of vascular endothelial growth factors and receptors in human periapical lesions. J Endod. 2013;39(5):605-11.

20. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N. Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod. 2015;41(6):797-803.

21. Storb R, Thomas ED. Allogeneic bone-marrow transplantation. Immunol Rev. 1983;71:77-102.

22. Deeg HJ, Socie G, Schoch G, Henry-Amar M, Witherspoon RP, Devergie A, et al. Malignancies after marrow transplantation for aplastic anemia and

fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood. 1996;87(1):386-92.

23. Bortin MM. A compendium of reported human bone marrow transplants. Transplantation. 1970;9(6):571-87.

24. Donato V, Iacari V, Zurlo A, Capua A, Tombolini V, Banelli E, et al. Fractionated total body irradiation in allogeneic bone marrow transplantation in leukemia patients: analysis of prognostic factors and results in 136 patients. Radiother Oncol. 1998;48(3):267-76.

25. Eun SC. Composite tissue allotransplantation immunology. Arch Plast Surg. 2013;40(2):141-53.

26. Forman SJ, Blume KG, Thomas ED. Hematopoietic cell transplantation: Blackwell Science; 1999.

27. Epstein JB, Chow AW. Oral complications associated with immunosuppression and cancer therapies. Infect Dis Clin North Am. 1999;13(4):901-23.

28. Gorr SU. Antimicrobial peptides in periodontal innate defense. Front Oral Biol. 2012;15:84-98.

29. Bishay N, Petrikowski CG, Maxymiw WG, Lee L, Wood RE. Optimum dental radiography in bone marrow transplant patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999;87(3):375-9.

30. Radmand R, Schilsky M, Jakab S, Khalaf M, Falace DA. Pre-liver transplant protocols in dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol. 2013;115(4):426-30.

31. Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology. 2010;139(4):1246-56, 56.e1-5.

32. Barbero P, Garzino Demo MG, Milanesio M, Ottobrelli A. [The dental assessment of the patient waiting for a liver transplant]. Minerva Stomatol. 1996;45(10):431-9.

33. Coates EA, Brennan D, Logan RM, Goss AN, Scopacasa B, Spencer AJ, et al. Hepatitis C infection and associated oral health problems. Aust Dent J. 2000;45(2):108-14.

34. Guggenheimer J, Eghtesad B, Close JM, Shay C, Fung JJ. Dental health status of liver transplant candidates. Liver Transpl. 2007;13(2):280-6.
35. Anand AC, Pardal PK, Sachdev VP. DENTAL CARIES AND PERIODONTAL DISORDERS IN CHRONIC LIVER DISEASE. Med J Armed Forces India. 2001;57(1):26-30.
36. Lins L, Bittencourt PL, Evangelista MA, Lins R, Codes L, Cavalcanti AR, et al. Oral health profile of cirrhotic patients awaiting liver transplantation in the Brazilian Northeast. Transplant Proc. 2011;43(4):1319-21.
37. Caly WR, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. J Hepatol. 1993;18(3):353-8.
38. Overholser CD, Peterson DE, Williams LT, Schimpff SC. Periodontal infection in patients with acute nonlymphocyte leukemia. Prevalence of acute exacerbations. Arch Intern Med. 1982;142(3):551-4.
39. Heimonen A, Janket SJ, Kaaja R, Ackerson LK, Muthukrishnan P, Meurman JH. Oral inflammatory burden and preterm birth. J Periodontol. 2009;80(6):884-91.
40. Scannapieco FA, Dasanayake AP, Chhun N. "Does periodontal therapy reduce the risk for systemic diseases?". Dent Clin North Am. 2010;54(1):163-81.
41. Fisher MA, Borgnakke WS, Taylor GW. Periodontal disease as a risk marker in coronary heart disease and chronic kidney disease. Curr Opin Nephrol Hypertens. 2010;19(6):519-26.
42. Offenbacher S, Barros SP, Beck JD. Rethinking periodontal inflammation. J Periodontol. 2008;79(8 Suppl):1577-84.
43. Guggenheimer J, Mayher D, Eghtesad B. A survey of dental care protocols among US organ transplant centers. Clin Transplant. 2005;19(1):15-8.

ARTIGOS CIENTÍFICOS

Dental Traumatology

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Assessment of the cytotoxicity of a mineral trioxide aggregate-based sealer with respect to macrophage activity

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Abstract – *Aim*: To assess the influence of co-culture with mineral trioxide aggregate (MTA) and MTA Fillapex (FLPX) on the viability, adherence, and phagocytosis activity of peritoneal macrophages from two mouse strains. *Methodology*: Cellular viability, adherence, and phagocytosis of *Saccharomyces boulardii* were assayed in the presence of capillaries containing MTA and MTA Fillapex. The data were analyzed using parametric (Student's *t*) and non-parametric (Mann–Whitney) tests. *Results*: FLPX was severely cytotoxic and decreased cell viability, adherence, and phagocytic activity of both macrophage subtypes. Cells that were treated with MTA Fillapex remained viable (>80%) for only 4 h after stimulation. Macrophages from C57BL/6 mice presented higher adherence and higher phagocytic activity compared with macrophages from BALB/c mice. *Conclusion*: Comparison of MTA and FLPX effects upon macrophages indicates that FLPX may impair macrophage activity and viability, while MTA seems to increase phagocytic activity.

Key words: *macrophage; mineral trioxide aggregate; MTA Fillapex; Viability; Adherence; phagocytosis*

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A complete sealing of the root canal system after cleaning and shaping is critical for a successful endodontic treatment. Root canals are traditionally filled with gutta-percha cones and a root canal sealer. It is highly desirable for sealers to be biocompatible because they can be placed in intimate contact with periapical tissues through the apical foramen and accessory communications (1). Moreover, sealers may release substances that may generate periapical inflammatory reactions.

Mineral trioxide aggregate (MTA) has been extensively studied and widely accepted for its biocompatibility and excellent sealing capacity (2–4). Research has demonstrated that MTA has better proprieties in terms of root repair and bone formation when compared with other commonly used material (5). Despite favorable characteristics, MTA does not present physical proprieties to be used as a sealer due to its long setting time and the difficulty of handing it in root canal. In an attempt to combine the physicochemical properties of the root canal sealer with the biological properties of MTA, an MTA-based sealer (MTA Fillapex®-Angelus; Londrina, Paraná, Brazil) was introduced to the market.

Macrophages are key cells for inflammation during chronic or healing processes (6). Macrophages were divided into two distinct cells types, M1 and M2, based on their receptor relationship, effector function, and cytokine production (7–12). M1 macrophages subtype typically produce high concentrations of interleukin IL-12 and low concentrations of IL-10, whereas M2 cells secrete high concentrations of IL-10 and low levels of the cytokine IL-12 (8, 9, 13, 14). Moreover, in pro-inflammatory M1 cells, high levels of inductive nitric oxide synthase (iNOS) result in citrulline and nitric oxide (NO) production. Conversely, in M2 cells, arginine metabolism leads to ornitine and urea production

that culminates in collagen synthesis and cell proliferation (10, 15, 16).

M1 macrophages mediate resistance to pathogens (17), while M2 macrophages are involved in healing process (7, 8, 10).

Despite few studies have analyzed the cytotoxicity of MTA Fillapex, no one has looked for its effects on macrophage viability and activity. This study assessed the influence of MTA and MTA Fillapex on M1 and M2 macrophages viability, adherence, and phagocytic activity.

Materials and methods

Mice

Male and female 4- to 8-week-old C57BL/6 and Balb/c mice were obtained from CEBIO (UFMG, Belo Horizonte, Brazil) and kept in a conventional animal house with barriers, temperature, and light control. Food and water were offered *ad libitum*.

Isolation of macrophages

Cells were isolated from the peritoneal cavity of C57BL/6 (M1 macrophages) and Balb/c (M2 macrophage) mice 5 days after injection of 2 ml of 3% thioglycolate medium (Biobrás S.A., Montes Claros, MG, Brazil) in the peritoneum. Cells were resuspended in complete medium: RPMI 1640 (Sigma Chemicals Co., St Louis, MO, USA), supplemented with 10% of fetal calf serum (Nutricell, Campinas, SP, Brazil), 0.1% of 0.05 mol l^{-1} β-mercaptoethanol (Sigma Chemicals Co.), 0.2% of penicillin (100 U ml^{-1})/streptomycin (0.1 mg ml^{-1}), and 200 mmol l^{-1} L-glutamine (18).

MTA and MTA Fillapex manipulation

MTA and MTA Fillapex® (FLPX) were prepared in accordance with manufactures instructions in sterile conditions. Soon after preparation, MTA and FLPX were inserted into the tips of previously sectioned sterilized capillary tubes (test group), so that their contact with the cell suspension could be standardized (18). Empty capillary tubes were used in control cultures.

Cell viability

The viability of cells was determined by two methods: the trypan blue exclusion assay and MTT assay. Cell viability assayed by trypan blue exclusion was performed in 24-well culture plates (2×10^5 cells ml^{-1} for 2, 4, 6, 8, 10, and 24 h) and in propylene tubes (1×10^6 cells ml^{-1} for 24 h), as described by others (3, 18). Briefly, cells were incubated in the presence of capillary tubes, in 1 ml of RPMI (Sigma Chemical Co.) containing 10% fetal calf serum (Nutricell, Campinas, SP, Brazil), 2 mM of L-glutamine and 100 units ml^{-1} of penicillin, and 100 μg ml^{-1} of streptomycin at 37°C and 5% CO2 humidified atmosphere. After the incubation periods, 100 μl of 0.25% trypan blue (Sigma Chemical Co.) in saline was added and cultures were examined under an inverted microscope. At least 300 cells were counted per culture (performed in triplicates), and results were expressed as percentage of viability. The experiment was repeated three times.

Additionally, cell viability was also determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich, St Louis, MO, USA).

The viability of cells in the presence of capillary tubes was tested by culturing 1×10^6 cells in 96-well culture plates for 24 h. 100 μl of RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, and streptomycin containing 1×10^6 cells/well and the capillaries tubes were seeded in 96-well plates and incubated for 24 h at 37°C. After 24 h, culture medium was removed, and the cells were gently washed with phosphate-buffered saline (PBS). A volume of 100 ml of MTT-succinate solution (1 mg ml^{-1}) was added to each well, and cells were incubated for an additional 4 h. The resulting formazan crystals were dissolved when removing the culture medium and adding 100 ml of dimethyl sulfoxide solvent (Sigma-Aldrich) to each well.

The absorbance was measured at 540 nm using a microplate reader ((Bio-Tek PowerWave HT USA, Winnooski, VT, USA). The formazan content of each well was computed as a percentage of the control group (19). The results were expressed as the percentage viability. The experiments were repeated three times in triplicate.

Cell adherence

Polypropylene tubes containing macrophages (1×10^6 cells ml^{-1}) were incubated for 2 h with capillaries (MTA, FLPX, and control groups) in an incubator with humidified atmosphere containing 5% CO_2, at 37°C. Tubes were agitated in a vortex agitator for 15 s, at low speed. Twenty microliters of the cellular suspension was removed, placed into a Newbauer chamber, and incubated for 1 h at 37°C as above. The percentage of adherent and non-adherent macrophages was then established by counting under an optical microscope (20).

Phagocytosis assay

Macrophages (1×10^6 cells ml^{-1}) were incubated for 2 h in 24-well culture plates (Nunclon, Nalge Nunc International, Miami, FL, USA), onto round glass coverslips (Glasstécnica São Paulo, SP, Brazil) in an incubator as above. Non-adherent cells were removed by washing with warm complete medium, afterward 10^7 CFU of *Saccharomyces boulardii* (Floratil; Merck S.A., Rio de Janeiro, RJ, Brazil) and capillaries with MTA and MTA Fillapex were added to the medium, and plates were incubated for 1 h. An empty capillary tube was added to control cultures. Unbound yeast cells were removed by washing with complete medium, and the coverslips were covered for 1 min with 1 ml of tannic acid at 1% (Merk, Billerica, MA, EUA), so that the distinction could be made between extracellular

and intracellular yeast cells. One drop of fetal calf serum was applied onto each coverslip. The dried coverslips were stained with Panótico Rápido (Laborclin Ltd, Pinhais, PR, Brazil) and glued to microscope glass slides with Entellan (Merk) for observation under optical microscope at 1000 magnification in oil immersion (21). Cells were counted until 200 macrophages with phagocyted yeast were found. The results were expressed in percentage.

Statistical analysis

Data were analyzed using parametric (Student's t) and non-parametric (Mann–Whitney) tests ($P < 0.05$). Analyses were made using the SPSS 18.0 Inc. (Statistical Package for Social Sciences, Chicago, IL, USA) software.

Results

Cell viability

Viability of cells is represented in Fig. 1. Control and test group cells in the presence of MTA remained viable for 24 h in the 24-well culture plates for both macrophages subtypes (Fig. 1a, b), as well in the propylene tubes assayed by trypan blue exclusion and in the 96-well culture plates assayed by MTT (data not shown). On the other hand, FLPX was severely cytotoxic and remarkably decreased cell viability when control was compared to test group for both macrophages ($P < 0.05$) (Fig. 1). However, M2 subtype survived better than M1 in the presence of FLPX ($P = 0.05$). Fig. 1c shows the viability curve of both macrophage signatures: cells treated with MTA Fillapex remained viable (>80%) only 4 h after stimulation. In this regard, cell viability assayed by MTT shows that, in FLPX group, more than 90% of cells died 24 h after capillaries exposure (data not shown). Very interesting is that MTA-treated cells show greater viability than control group for both macrophage subtypes ($P \leq 0.021$) when cell viability was assayed by MTT (data not shown).

Cell adherence

M1 macrophages, in control or MTA group, presented higher adherence ability than M2 macrophage ($P = 0.02$) (Fig. 2). However, cell adherence of both

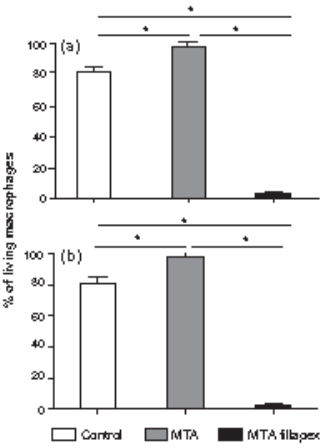

Fig. 2. The percentage of living macrophages (a) from C57BL/6 mice (a) and macrophages from BALB/c mice (b), after MTT assay with capillaries containing MTA or MTA Fillapex. The controls were cultured with empty capillaries. The cultures were maintained for 24 h as described in Materials and Methods. Bars represent the mean of three experiments; lines represent the standard error of the means. *indicates a statistically significant difference between groups ($P < 0.05$).

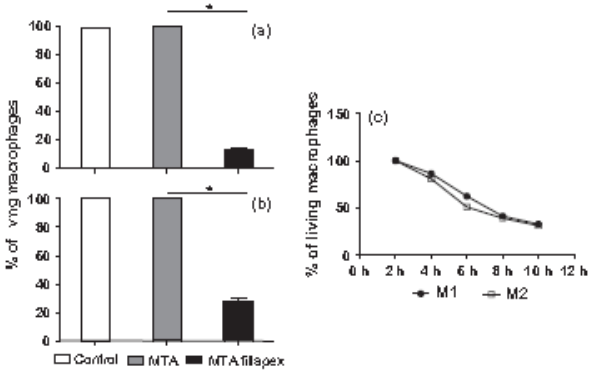

Fig. 1. The percentage of living macrophages (a) from C57BL/6 mice and macrophages (b) from BALB/c mice, after incubation of the culture plates with capillaries containing MTA or MTA Fillapex. The controls were cultured with empty capillaries. The cultures were maintained for 24 h as described in Materials and Methods. Bars represent the mean of three experiments; lines represent the standard error of the means. (c) The percentage of living macrophages from C57BL/6 and from BALB/c treated with MTA Fillapex at different time points. *indicates a statistically significant difference between groups ($P < 0.05$).

macrophages subtypes is impaired by FLPX ($P < 0.05$) when compared to MTA and control groups.

Phagocytic activity

Herein, it was assayed the capacity of mouse peritoneal cells to uptake yeast cells (Fig. 3). When M1 and M2 control groups were compared, the M1 macrophages showed statistically higher phagocytic ability than M2 macrophages ($P = 0.033$), in spite of cells being treated or not. However, phagocytosis activity of *S. Boulardii* was statistically impaired in the presence of FLPX in both macrophages subtypes ($P < 0.05$), as well as the percentage of M1 cells with ingested yeast was higher than M2 cells ($P < 0.05$).

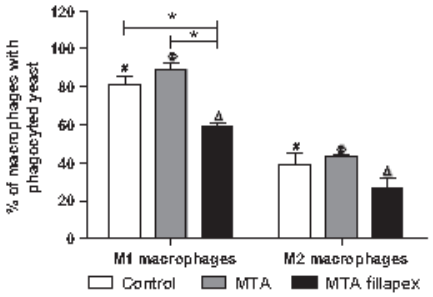

Fig. 4. The percentage of macrophages from C57BL/6 mice (a) and macrophages from BALB/c mice (b) that were observed to phagocytose yeast. The controls were cultured with empty capillaries. The cultures were performed as described in Materials and Methods. Bars represent the mean results of two experiments performed in triplicate. Lines indicate the standard error of the means. *indicates a statistically significant difference between groups ($P < 0.05$). #, Φ, and Δ indicate significant differences between macrophage sources ($P < 0.05$).

Discussion

Successful root canal treatment depends on the elimination of intracanal infection, followed by effective and biocompatible canal filling to avoid reinfection and irritation of the periradicular tissue (22). Then, biocompatibility of the endodontic root canal sealer may be one of its main requirements (23, 24).

Macrophages prevail in inflammatory infiltrates in response to sealers, along with lymphocytes and plasma cells (25). Major functions of macrophages include elimination of invading bacteria, recruitment of other cells to the site of infection, clearance of the excess neutrophils, production of cytokines and chemokines, and activation of the lymphocyte-mediated adaptive immune response (6). Macrophages, together with neutrophils, are responsible of phagocytosis and digestion of microorganisms and foreign substances through surface receptors that recognize and bind certain surface molecules of bacteria such as the lipopolysaccharides (26).

Using a well-tested methodology (3, 12, 18), the present study evaluated the macrophage immune response to MTA Fillapex and compared these results with those of MTA. The viability, adherence, and phagocytic activity of M1 and M2 macrophages were assayed. These macrophages differ in terms of their inherent receptors, expression of cytokines and chemokines, and effector functions, such that the M1 macrophages are microbicidal and inflammatory and the M2 macrophages are immunomodulatory and poorly microbicidal (3, 7).

Macrophages were exposed to manipulated MTA Fillapex and MTA *in vitro*, using a previously described system (18) that allows a controlled exposure of cell cultures to the sealer and MTA. FLPX remarkably decrease cell viability in all conditions tested. Accordingly, other studies have reported that FLPX reduced the cell survival rates (27–29). To explain this unexpected FLPX behavior, it is necessary to realize that it contains toxic components, as salicylate resin. Previous studies evaluated the effect of salicylate resin on human fibrosarcoma cells line (HT-1080) and observed cellular apoptosis after resin salicylate stimuli (30, 31).

In this study, the superb cell viability promoted by MTA when compared with control cells was observed for both macrophage subtypes (M1 and M2), as previously described (3). Similar MTA effects were reported concerning fibroblasts (32, 33), osteoblasts (34, 35) and

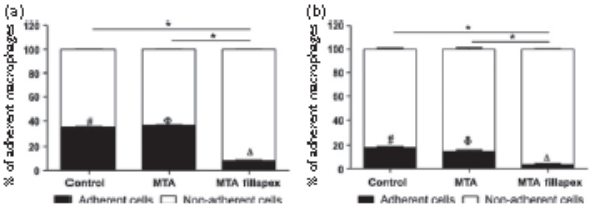

Fig. 3. The percentage of adherent macrophages from C57BL/6 mice (a) and macrophages from BALB/c mice (b), after incubation in polypropylene tubes with capillaries containing MTA and MTA Fillapex. The controls were cultured with empty capillaries. The cultures were performed as described in Materials and Methods. Bars represent the mean results of four experiments performed in duplicate. Lines indicate the standard error of the means. *indicates a statistically significant difference between groups ($P < 0.05$). #, Φ, and Δ indicate significant differences between macrophage sources ($P < 0.05$).

macrophages cells (3, 11, 36). Conversely, cells treated by FLPX remained viable (>80%) for only 4 h (Fig. 1c). This short time of cell viability determined our next experiments with macrophages adherence and phagocytosis. To perform them, cells were incubated for 2 h, when the viability of macrophage-treated by FLPX was high yet.

Adherence is the first step of the phagocytic process (37). Several studies that evaluate biocompatibility of endodontic materials assayed macrophage adherence and spreading (18, 38). Here, MTA Fillapex decreased the ability of M1 and M2 macrophages to adhere to glass, clearly showing that this sealer is able to impair immune system responses. Accordingly, it has being shown lower cell adherence values in the presence of ZOE sealers (12, 18, 39), as well as with other endodontic materials (40–42). On the other hand, MTA did not affect macrophage adherence, as previously described (3). Similarly, MTA did not interfere in adherence of osteoblasts and an osteosarcoma cell line (34, 38). Interestingly, when M1 and M2 control groups were compared, M1 macrophages show statistically higher adherence values than M2 ones.

Root canal sealers are placed in intimate contact with periapical tissues that might be inflamed and/or infected, and it is fundamental that it does not interfere with the host's phagocytosis process. In this study, the capability of both macrophage subtypes to ingest *Saccharomyces boulardii* was evaluated in the presence of MTA Fillapex and MTA. This yeast was selected because of its size, which makes counting easier, and therefore allows greater data precision (3). The M1 macrophages show statistically higher phagocytic ability than M2 macrophages in all groups tested. Consistent with this result, (12) have reported that M2 macrophages treated by two different zinc oxide–eugenol-based materials also had their phagocytic activity of *S. boulardii* reduced.

Conclusion

It has recently been reported that MTA does not interfere in the immune responses by M1 or M2 macrophages (3, 11). On the other hand, the endodontic MTA-based sealer tested in this study, MTA Fillapex, was severely cytotoxic and remarkably decreases M1 or M2 macrophages viability. Moreover, FLPX also impaired cell adhesion properties and the phagocytic ability of both macrophages subtypes. The findings of this study infer that MTA Fillapex may decrease the ability of macrophages and interfere with the healing of an endodontic treatment. The composition needs to be re-evaluated in future studies to determine what component or combination or components may be causing the negative effects. In addition, further animal or *in vivo* studies are required to determine whether the outcome of treatment is affected.

Acknowledgements

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References

1. Bernath M, Szabo J. Tissue reaction induced by different sealers. Int Endod J 2003;36:256–61.
2. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25:197–205.
3. Rezende TM, Vieira LQ, Cardoso FP, Oliveira RR, de Oliveira Mendes ST, Jorge ML. The effect of mineral trioxide aggregate on phagocyte activity and production of reactive oxygen, nitrogen species and arginase activity by M1 and M2 macrophages. Int Endod J 2007;40:603–11.
4. Scarparo RK, Haddad D, Acosigua GA, Fossati AC, Fachin EV, Grecca FS. Mineral trioxide aggregate based sealer: analysis of tissue reactions to a new endodontic material. J Endod 2010;36:1174–8.
5. Torabinejad M, Pitt Ford TR, Abedi HR, Kariyawasam SP, Tang HM. Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. J Endod 1998;24:468–71.
6. Hasturk H, Kantarci A, Van Dyke TE. Oral inflammatory diseases and systemic inflammation: role of the macrophage. Front Immunol 2012;3:118.
7. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 2000;164:6166–73.
8. Bastos KR, Alvarez JM, Marinho CR, Rizzo LV, Lima MR. Macrophages from IL-12p40-deficient mice have a bias toward the M2 activation profile. J Leukoc Biol 2012;71:271–8.
9. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–55.
10. Mosser DM. The many faces of macrophage activation. J Leukoc Biol 2003;73:209–12.
11. Rezende TM, Vargas DL, Cardoso FP, Sobrinho AP, Vieira LQ. Effect of mineral trioxide aggregate on cytokine production by peritoneal macrophages. Int Endod J 2005;38:896–903.
12. de Oliveira Mendes ST, Brito LCN, Rezende TMB, Reis RO, Cardoso FP, Vieira LQ et al. A decrease in innate immune response to infection in the presence of root canal sealers. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:315–23.
13. Edwards JP, Zhang X, Frauwirth KA, Mosser DM. Biochemical and functional characterization of three activated macrophage populations. J Leukoc Biol 2006;80:1298–307.
14. Verreck FA, De Boer T, Langenberg DM, Hoeve MA, Kramer M, Vaisberg E et al. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. Proc Natl Acad Sci 2004;101:4560–5.
15. Bogdan C, Vodovotz Y, Nathan C. Macrophage deactivation by interleukin 10. J Exp Med 1991;174:1549–55.
16. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. Nat Rev Immunol 2005;5:641–54.
17. Nathan C, Shiloh MU. Reactive oxygen and nitrogen intermediates in the relationship between mammalian hosts and microbial pathogens. Proc Nath Acad Sci USA 2000;97:8841–8.
18. de Oliveira Mendes ST, Ribeiro Sobrinho AP, de Carvalho AT, de Souza Cortes MI, Vieira LQ. In vitro evaluation of

the cytotoxicity of two root canal sealers on macrophage activity. J Endod 2003;29:95–9.
19. van de Loosdrecht AA, Nennie E, Ossenkoppele GJ, Beelen RH, Langenhuijsen MM. Cell mediated cytotoxicity against U 937 cells by human monocytes and macrophages in a modified colorimetric MTT assay. A methodological study. J Immunol Methods 1991;141:15–22.
20. Lee A, Whyte MK, Haslett C. Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. J Leukoc Biol 1993;54:283–8.
21. Gamms J, Lombard Y, Makaya Kumba M, Fonteneau P, Pondron P. A new and simple method for studying the binding and ingestion steps in the phagocytosis of yeasts. J Immunol Methods 1992;154:185–93.
22. Ricucci D, Siqueira JF Jr. Fate of the tissue in lateral canals and apical ramifications in response to pathologic conditions and treatment procedures. J Endod 2010;36:1–15.
23. Al Hiyasat AS, Tayyar M, Darmani H. Cytotoxicity evaluation of various resin based root canal sealers. Int Endod J 2010;43:148–53.
24. López López J, Esturgo Devesa A, Jané Salas E, Segura Egea JJ. Inferior alveolar nerve injury resulting from overextention of an endodontic sealer: non surgical management using GABA analogue pregabalin. Int Endod J 2012;45:98–104.
25. Orstavik D, Myór IA. Usage test of four endodontic sealers in *Macaca fascicularis* monkeys. Oral Surg Oral Med Oral Pathol 1992;73:337–44.
26. Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of an on clonal system of recognition. Cell 1997;91:295–8.
27. Bin CV, Valera MC, Camargo SEA. Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. J Endod 2012;38:495–500.
28. Souza MZ, Linhares AB, da Silva LE, Granjeiro JM, Alves GG. A multiparametric assay to compare the cytotoxicity of endodontic sealers with primary human osteoblasts. Int Endod J 2012;45:12–8.
29. Silva EJNL, Rosa TP, Herrera DR, Jacinto RC, Gomes BPFA, Zaia AA. Evaluation of Cytotoxicity and Physicochemical Properties of Calcium Silicate based Endodontic Sealer MTA Fillapex. J Endod 2013;39:274–7.
30. Stark LA, Din FV, Zwacka RM, Dunlop MG. Aspirin induced activation of the NFkappaB signaling pathway: a novel mechanism for aspirin mediated apoptosis in colon cancer cells. FASEB J 2001;15:1273–5.
31. Mahdi JG, Alkarrawi MA, Mahdi AJ, Bowen ID, Human D. Calcium salicylate mediated apoptosis in human HT 1080 fibrosarcoma cells. Cell Prolif 2006;39:249–60.
32. Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod 2000;26:288–91.
33. Saidon J, He J, Zhu Q, Safavi K, Spangberg LS. Cell and tissue reactions to mineral trioxide aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:483–9.
34. Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to mineral trioxide aggregate. J Endod 1998;24:543–7.
35. Mitchell PJ, Pitt Ford TR, Torabinejad M, McDonald F. Osteoblast biocompatibility of mineral trioxide aggregate. Biomaterials 1999;20:167–73.
36. Haglund R, He J, Jarvis J, Safavi KE, Spangberg LS, Zhu Q. Effects of root end filling materials on fibroblasts and macrophages in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:739–45.
37. Unanue ER, Allen PM. The immunoregulatory role of the macrophage. Hosp Pract (Off Ed) 1987;22:87–98, 102–4.
38. Zhu Q, Haglund R, Safavi KE, Spangberg LS. Adhesion of human osteoblasts on root end filling materials. J Endod 2000;26:404–6.
39. Sadeghein A, Bolhari B, Sarafnejad A. A comparison of the effect of three endodontic sealers on adherence of mouse peritoneal macrophages. J Calif Dent Assoc 2001;29:673–7.
40. Segura JJ, Calvo JR, Guerrero JM, Sampedro C, Jiménez A, Llamas R. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: endodontic implications. J Endod 1996;22:337–40.
41. Jiménez Rubio A, Segura JJ, Llamas R, Jiménez Planas A, Guerrero JM, Calvo JR. In vitro study of the effect of sodium hypochlorite and glutaraldehyde on substrate adherence capacity of macrophages. J Endod 1997;23:562–4.
42. Segura JJ, Jiménez Rubio A. Effect of eugenol on macrophage adhesion in vitro to plastic surfaces. Endod Dent Traumatol 1998;14:72–4.

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The effects of a mineral trioxide aggregate-based sealer on the production of reactive oxygen species, nitrogen species and cytokines by two macrophage subtypes

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Abstract

Braga JM, Oliveira RR, Martins RC, Ribeiro Sobrinho AP. The effects of a mineral trioxide aggregate based sealer on the production of reactive oxygen species, nitrogen species and cytokines by two macrophage subtypes. *International Endodontic Journal*.

Aim To test the effects of a mineral trioxide aggregate-based sealer (MTA Fillapex®) and MTA (MTA-Ângelus®) on viability and on the production of cytokines, reactive oxygen species (ROS) and nitrogen species (NO) by M1 and M2 inflammatory macrophages.

Methodology M1 (from C57BL/6 mice) and M2 (from BALB/c mice) peritoneal inflammatory macrophages were obtained and cultured *in vitro* in the presence of original and diluted extracts of MTA and MTA Fillapex (FLPX). The cell viability, ROS release and the release of tumour necrosis factor-a, interleukin (IL)-12, IL-10 and NO in response to stimulation with interferon-γ and *Fusobacterium nucleatum* or *Peptostreptococcus anaerobius* were evaluated. The data were analysed using the Mann–Whitney test and Student's *t*-test.

Results Fillapex was cytotoxic at the highest concentrations (1 : 1;1 : 2) and decreased the viability ($P < 0.05$) of both macrophage types (<20%). MTA did not interfere with cellular viability. FLPX inhibited the release of ROS and decreased NO release in *F. nucleatum* and *P. anaerobius* -stimulated M1 and M2 macrophages (≤25 μ mol L^{-1}). *F. nucleatum*–stimulated M2 macrophage cultures released lower levels of TNF-α when FLPX was added (≤1 ng mL^{-1}). M2 macrophages released higher (>5 ng mL^{-1}) levels of IL-10 than M1 macrophages. Only M1 macrophage cultures produced IL-12p70.

Conclusions Fillapex impaired effector immune responses during inflammation (M1 macrophages), as well as during healing (M2 macrophages) responses.

Keywords: biocompatibility, cytokines, macrophages, MTA and MTA Fillapex sealer, nitrogen species, reactive oxygen species.

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Introduction

Successful root canal treatment consists of cleaning and shaping procedures followed by filling of the root canal with gutta-percha and sealer. Independent of the physicochemical properties of the sealers, if these materials are not biocompatible, it can cause degeneration of the periapical tissue and delay wound healing (Murray *et al.* 2007, Sousa *et al.* 2009). Recently, MTA Fillapex (Angelus, Londrina, PR, Brazil), an MTA-based sealer, was introduced in the market in an attempt to combine the physicochemical properties of a root canal sealer with the biological properties of Mineral Trioxide Aggregate (MTA) (Torabinejad &

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Chivian 1999, Rezende *et al.* 2007, Scarparo *et al.* 2010). It has been demonstrated that MTA has better properties in terms of root repair and bone formation when compared with other commonly used materials such as intermediate restorative material (IRM; Dentsply, Milford, DE, USA), glass ionomer and reinforced zinc oxide–eugenol cement (Super-EBA; Harry J. Bosworth Company, Skokie, IL, USA) (Torabinejad *et al.* 1995, 1998). Previously, it has been shown that MTA does not affect the activity of macrophages. More specifically, MTA did not effect the antibacterial activities of either M1- or M2-type macrophages, including bacterial phagocytosis, reactive oxygen and nitrogen species production or arginase activity (Rezende *et al.* 2005). It has also been shown that MTA upregulated the adaptive humoral immune responses but had little or no effect on pro- or anti-inflammatory cytokine production by memory T cells (Rezende *et al.* 2008).

Frequently, root canal sealers extrude or contact periapical tissues beyond the apical foramen, and these sealers can occasionally cause tissue inflammation. In this latter condition, the recruitment of inflammatory cells and the release of pro-inflammatory and immune regulatory cytokines have occurred (Stashenko *et al.* 1998, de Brito *et al.* 2012). Macrophages predominated among the cells that were recruited to this area (Van Dyke 2008, Hasturk *et al.* 2012). The main functions of macrophages include the elimination of invading bacteria, recruitment of other cells to the site of infection, clearance of excess neutrophils, production of cytokines and chemokines and activation of the lymphocyte-mediated adaptive immune response (Taylor *et al.* 2005, Hasturk *et al.* 2012).

Macrophages were divided into the M1 and M2 subtypes based on their receptor relationship, effector function and cytokine production (Mills *et al.* 2000, Bastos *et al.* 2002, Rezende *et al.* 2005, de Oliveira Mendes *et al.* 2010). The M1 macrophage subtype typically produces high concentrations of interleukin (IL)-12 and low concentrations of IL-10, whereas M2 cells secrete high concentrations of IL-10 and low levels of IL-12 (Bastos *et al.* 2002, Mantovani *et al.* 2002, Verreck *et al.* 2004, Edwards *et al.* 2006). Moreover, in pro-inflammatory M1 cells, high levels of inductive nitric oxide synthase (iNOS) result in citrulline and nitric oxide (NO) production. In M2 cells, arginine metabolism leads to ornithine and urea production, which culminates in collagen synthesis and cell proliferation (Bogdan *et al.* 1991, Mosser 2003, Bronte & Zanovello 2005).

The aim of this study was to determine the effects of MTA Fillapex (Angelus, Londrina, PR, Brazil) and white MTA (Angelus) on M1 and M2 macrophage viabilities. Additionally, the effects of these materials on cytokine production and the release of reactive oxygen species and nitrogen species by both macrophage types were assessed. The null hypothesis was that the association of the endodontic sealer with MTA did not affect macrophage responses.

Materials and methods

Mice

Male and female C57BL/6 and BALB/c mice between 4–8 weeks of age were obtained from CEBIO (UFMG, Belo Horizonte, Brazil) and kept in a conventional animal house with barriers, temperature and light control. Food and water were offered *ad libitum*.

Isolation of macrophages

Cells were isolated from the peritoneal cavity of 12 C57BL/6 (M1 macrophages) and 12 BALB/c (M2 macrophage) mice 5 days after peritoneal injection of 2 mL of 3% thioglycolate medium (Biobrás S.A., Montes Claros, MG, Brazil). Mice were killed by decapitation, exsanguinated, and 10 mL of sterile RPMI medium (Sigma Chemical Co., St. Louis, MO, USA) were quickly injected into the peritoneal cavity using a syringe and an 18-gauge needle. Cells were recovered from the peritoneal cavity by aspirating back the medium containing the inflammatory cells. Over 90% of the recovered cells had morphological characteristics of macrophages when visualized by optical microscopy. Cells were resuspended in complete medium: RPMI 1640 (Sigma Chemicals Co.), supplemented with 10% foetal calf serum (Nutricell, Campinas, SP, Brazil), 0.1% of 0.05 mol L^{-1} β-mercaptoethanol (Sigma Chemicals Co.), 0.2% penicillin (100 U mL^{-1})/streptomycin (0.1 mg mL^{-1}) and 200 m mol L^{-1} L-glutamine (de Oliveira Mendes *et al.* 2003). The experimental protocol was approved by the animal ethics committee (101/2012, CETEA/UFMG).

Preparation of extracts

MTA and MTA Fillapex® (FLPX) were manipulated under sterile conditions according to manufactures' instructions and inserted into sterile 16.2-mm-diameter 24-well plates, immediately after mixing.

Subsequently, the plates were incubated at 37 °C for 24 h. Afterwards, specimens were then covered with 2.5 mL RPMI 1640 (Sigma Chemicals Co.) supplemented with 10% foetal bovine serum, penicillin, and streptomycin and incubated for 24 h at 37 °C. After incubation, these original extracts (1 : 1) were then serially diluted (1 : 2, 1 : 4, 1 : 8, 1 : 16 and 1 : 32) in cell culture medium before testing.

Cytotoxicity testing

Viability of cells in the presence of extracts, in original or serial dilutions, was tested by culturing 1×10^6 cells in 96-well culture plates for 24 and 72 h, in 100 μL of RPMI 1640 supplemented with 10% foetal bovine serum, penicillin and streptomycin at 37 °C. The cells were exposed to 200 μL of the extracts in a total volume of 300 μL. After 24 h, cell survival was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich) (van de Loosdrecht *et al.* 1991). The results were expressed as the percentage of viable cells. Experiments were reproduced in triplicate.

Microorganism preparation

To induce tumour necrosis factor (TNF), reactive oxygen species (ROS), IL-12, IL-10 and NO, root canal pathogens were selected: *Fusobacterium nucleatum* (ATCC 10953), a gram-negative bacterium, and *Peptostreptococcus anaerobius* (ATCC 27337), a gram-positive bacterium. Zymosan A from *Saccharomyces cerevisiae* (Sigma Chemical Co.) was used as a positive control for the ROS assay (Rezende *et al.* 2007).

The microorganisms were grown in brain heart infusion broth (BHI; Difco, Detroit, MI, USA), supplemented and pre-reduced (BHI-PRAS) in an anaerobic chamber (Forma Scientific, Marietta, OH, USA) containing an atmosphere composed of 85% N_2, 10% H_2 and 5% CO_2 for 48 h at 37 °C. The samples were adjusted in phosphate-buffered saline (PBS) to a concentration of 8×10^7 CFU mL^{-1}. To kill the bacteria, the methodology proposed by Ribeiro Sobrinho *et al.* (2002) was used. Zymosan A was diluted in PBS (10^9 particles mL^{-1}) (Trush *et al.* 1978).

Reactive oxygen intermediates assay

Reactive oxygen species were assayed as described by Trush *et al.* (1978) with slight modifications. Macrophages (1×10^6 cells/well) were transferred to a C96 White Maxisorp (Nalgene, Rochester, NY, USA) plate in a total volume of 200 μL that contained: 0.05 m mol L^{-1} luminol, diluted extract (1 : 4) and 10^7 zymosan particles (Sigma Chemical Co.) in RPMI 1640 without phenol red. Diluted extract was not used in control groups. The plates were read in a luminometer every 2 min for a total of 118 min (LumiCount Packard Instrument Company, Downers Grove, IL, USA). The results were expressed as the area under each of the curves. The experiments were performed three times in triplicate.

Cell culture and cytokine assays

Fusobacterium nucleatum and *Peptostreptococcus anaerobius* (10^7 UFC mL^{-1}) preparations were added to 24-well plates (Nunclon) containing a peritoneal cell suspension (2×10^6 cells/well) and the diluted extract (1 : 4) in the presence or absence of 10 U mL^{-1} of recombinant murine interferon (IFN)-γ (Pharmingem, San Diego, CA, USA), in a final volume of 1 mL. Plates were incubated for 24 h to assay for TNF and IL-12 production and for 72 h to assay for IL-10 and NO production. Supernatants were harvested, and cytokine readings were performed. The experiments were performed two times in quadruplicate to assay the IL-10, IL-12 and TNF production and three times in triplicate to assay NO production.

Statistical analysis

The data were analysed using parametric (Student's *t*-test) and nonparametric (Mann–Whitney) tests ($P < 0.05$). Analyses were carried out using SPSS 18.0 Inc. software (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

Cell Viability

Cell viability using MTT is demonstrated in Fig. 1. When the control group was compared to the test group, FLPX sealer was cytotoxic at the highest concentrations (1 : 1, 1 : 2) and significantly decreased the viability of both macrophage types (<20%) at 24 h and 72 h after stimulation ($P < 0.05$). However, cells treated with diluted FLPX extracts (1 : 4, 1 : 8) remained viable (>80%) for 24 h and 72 h. (Fig. 1a, b). Macrophages of both subtypes remained viable (>80%) after stimulation with diluted MTA extracts

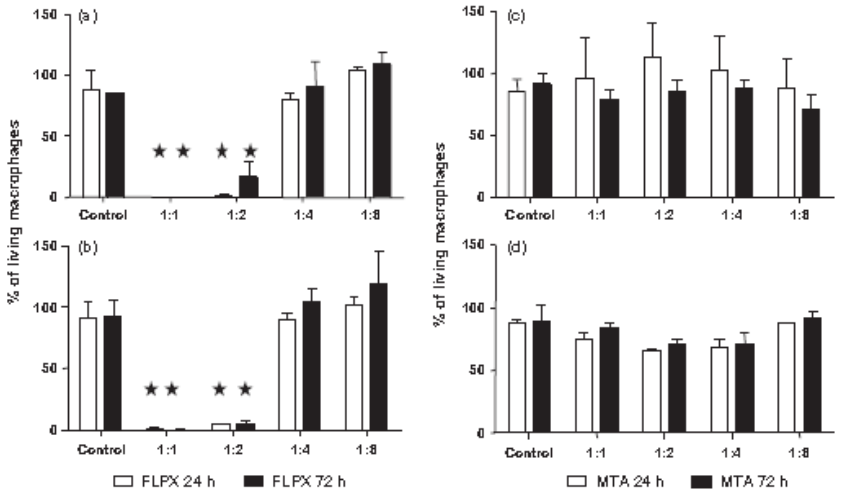

Figure 1 Percentage of living M1 macrophages (a and c) and M2 macrophages (b and d) after exposure to extracts and organized according to type of dilution. Cell cultures were exposed to original extracts (1 : 1) and to serially diluted extracts for 24 h □ and for 72 h ■. Columns represent the mean cell viability expressed as percentages. Black stars (★) indicate significant differences between treatment with original and diluted extracts.

(1 : 1, 1 : 2, 1 : 4 and 1 : 8) (Fig. 1c,d). No differences were found between the viabilities of M1 and M2 macrophages.

ROS assay

Analysis of the area under each ROS production curve showed that zymosan stimulated significantly higher respiratory bursts ($P < 0.05$) for M2 macrophages (>200 000 RLU) compared to M1 macrophages (Fig. 2). Fig. 2 demonstrates that FLPX sealer inhibited the release of ROS by M1 and M2 macrophages ($P \leq 0.05$). Additionally, FLPX sealer significantly decreased ROS release (≤50 000 RLU) in zymosan-stimulated cultures ($P < 0.0001$). MTA treatment did not affect ROS release by M2 macrophages; however, lower levels of ROS were observed in zymosan-stimulated M1 macrophages (<50 000 RLU) compared to the control (>50 000 RLU) group ($P < 0.004$).

NO release

M1 and M2 macrophages released similar levels of NO in all of the conditions examined. The only exception was observed in *Peptostreptococcus anaerobius*-stimulated M2 macrophages, where high amounts of NO (>30 μ mol L^{-1}) were observed ($P \leq 0.046$). NO release by M1 and M2 cells was increased when IFN-γ was added to the cultures (Fig. 3). The addition of *Fusobacterium nucleatum*-stimulated cells induced NO release, and these levels were higher when IFN-γ was added to the culture. MTA decreased NO release (<150 μ mol L^{-1}) in M1 *F. nucleatum*-stimulated cultures with IFN-γ stimuli ($P \leq 0.001$). As observed for ROS, FLPX sealer decreased NO release (<20 μ mol L^{-1}) in *F. nucleatum* and *P. anaerobius* -stimulated M1 and M2 macrophages ($P < 0.05$).

TNF-α

M2 macrophages released higher levels of TNF-α compared to M1 macrophages in all of the conditions tested ($P \leq 0.05$) (Fig. 4). FLPX sealer induced TNF-α release (>0.5 ng mL^{-1}) in *P. anaerobius*-stimulated M1 macrophage cultures in the presence of IFN-γ ($P \leq 0.05$) (Fig. 4a). TNF-α release by *F. nucleatum*-stimulated M1 cells was not influenced by the presence of FLPX sealer. However, FLPX sealer decreased

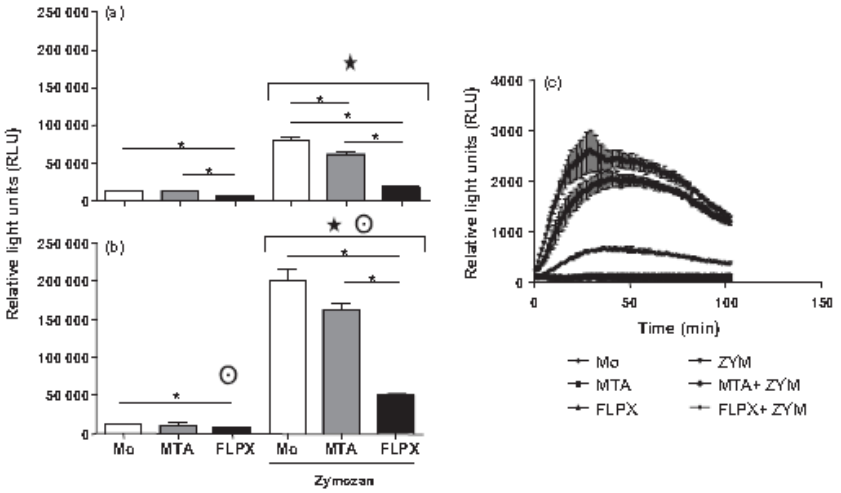

Figure 2 Mean production of reactive oxygen intermediates (ROS) by M1 macrophages (a) and M2 macrophages (b). Representative kinetics of ROS production by zymosan-stimulated and unstimulated M2 macrophages in the presence or absence of sealers are shown in (c). Cells were cultured with diluted extracts of MTA and FLPX sealer (1 : 4) and stimulated with zymosan. * indicates a statistically significant difference between groups ($P < 0.05$). Black stars (★) indicate significant differences between control and stimulated groups, and white circles (○) indicate significant differences between macrophage sources ($P < 0.05$).

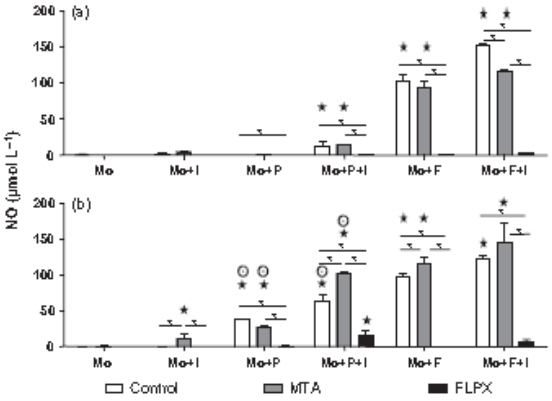

Figure 3 Nitric oxide (NO) production by M1 macrophages (a) and M2 macrophages (b). Cells were cultured with diluted extracts of MTA and FLPX (1 : 4) and stimulated with *F. nucleatum* (F) or *P. anaerobius* (P), and IFN-gamma (I). * indicates a statistically significant difference between groups ($P < 0.05$). Black stars (★) indicate significant differences between control and stimulated groups, and white circles (○) indicate significant differences between macrophage sources ($P < 0.05$).

TNF-α release (<1.0 ng mL $^{-1}$) in the presence or absence of IFN-γ in *F. nucleatum*-stimulated M2 macrophages ($P \leq 0.05$) (Fig. 4b).

IL-12p70

IL-12p70 release was detected only in M1 macrophages. FLPX sealer did not interfere with IL-12p70 release in any condition as compared with the control groups. An exception was observed in *P. anaerobius*-stimulated cells, which increased IL-12p70 release (>0.5 ng mL $^{-1}$) in the presence of IFN-γ ($P < 0.05$). MTA significantly stimulated IL-12p70 release by M1 macrophages in all conditions ($P < 0.05$) except for *F. nucleatum*-stimulated cultures (Fig. 5).

Il-10

Figure 4 shows that M2 macrophages released higher levels of IL-10 than M1 macrophages in all conditions

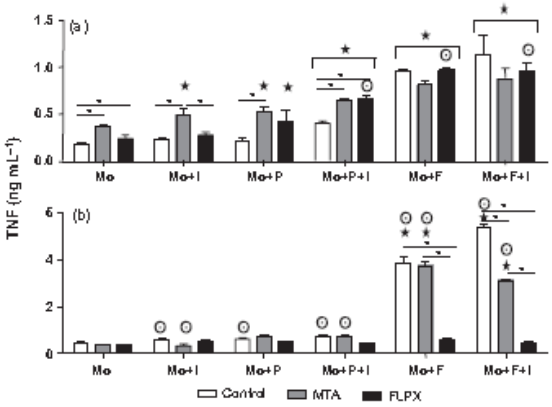

Figure 4 Mean production of TNF by M1 (a) and M2 (b) macrophages cultured in the absence (control) or presence of sealer extracts (1 : 4) 24 h after incubation. The cells were cultured in the medium alone (M control), or with *Fusobacterium nucleatum* (F) or *Peptostreptococcus anaerobius* (P) preparations. Interferon-γ (I) was added where indicated. * indicates a statistically significant difference between groups ($P < 0.05$). Black stars (★) indicate significant differences between control and stimulated groups, and white circles (○) indicate significant differences between macrophage sources ($P < 0.05$).

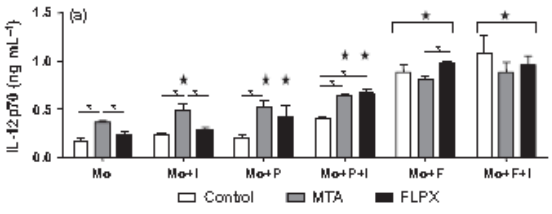

Figure 5 Mean production of IL-12 by M1 (a) macrophages cultured in the absence or presence of diluted sealer extracts (1 : 4) 24 h after incubation. The cells were cultured in medium alone (M control), or with *Fusobacterium nucleatum* (F) or *Peptostreptococcus anaerobius* (P) preparations. Interferon-γ (I) was added where indicated. M2 macrophages did not produce IL-12. Moreover, * indicates a statistically significant difference between groups ($P < 0.05$). Black stars (★) indicate significant differences between control and stimulated groups ($P < 0.05$).

tested ($P < 0.05$). FLPX sealer stimulated the release of IL-10 by M1 and M2 macrophages in *P. anaerobius*-stimulated macrophages cultured in the presence or absence of IFN-γ. MTA also stimulated IL-10 release (>5.0 ng mL ^{1}) by M2 macrophages in *P. anaerobius* and *F. nucleatum* -stimulated cultures (Fig. 6). M2 macrophages that were pre-incubated with MTA extract and subsequently treated with *F. nucleatum* antigen in the absence of IFN-δ released the highest levels of IL-10 (>15.0 ng mL ^{1}) ($P < 0.05$) (Fig. 6b).

Discussion

Studies to assay the effects of root canal sealers on macrophage function provide important knowledge about the innate and adaptive immune responses in inflamed periapical tissues surrounding teeth subjected to endodontic treatments (Perassi *et al.* 2004, Rezende *et al.* 2005, Sousa *et al.* 2009, de Oliveira Mendes *et al.* 2010). Professional antigen-presenting cells (APCs) such as macrophages ingest foreign particles, including infectious agents and cellular debris (Kopitar-Jerala 2006, Hasturk *et al.* 2012). Several studies have shown that endodontic materials can impair the phagocytic activities of periapical APCs (Lee *et al.* 2007, Sousa *et al.* 2009, de Oliveira Mendes *et al.* 2010, Brackett *et al.* 2011).

In this study, a few parameters of the innate immune response (M1 and M2 macrophage responses) to MTA Fillapex, a new root canal sealer, and MTA were analysed. Moreover, their response in the presence of root canal pathogens was also assayed. The macrophage response to sealers during the inflammation (M1 macrophages) and healing (M2 macrophages) processes was also explored.

The first step was to analyse cell viability in the presence of sealers. As previously shown (Rezende *et al.* 2005), MTA did not affect M1 or M2 macrophage viability. However, FLPX sealer significantly decreased cell viability of both macrophage subtypes after stimulation with high extract concentrations (1 : 1, 1 : 2). Bin *et al.* (2012) observed similar results when using original and serially diluted extracts of these sealers. Several other groups have confirmed these findings (Salles *et al.* 2012, Scelza *et al.* 2012, Silva *et al.* 2013). MTA Fillapex (Angelus) contains toxic components, such as salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, pigments, which could be responsible for the negative outcomes observed (Stark *et al.*2001, Mahdi *et al.* 2006, Faria-Júnior *et al.* 2013). After analysing these findings, it was determined that the best concentration of FLPX and MTA was 1–4 (1 : 4) because this ratio did not interfere with macrophage viability.

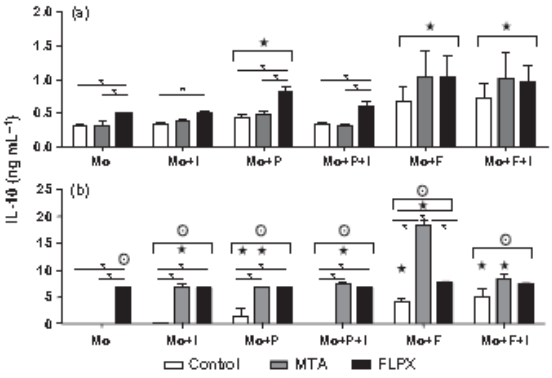

Figure 6 Mean production of IL-10 by M1 (a) and M2 (b) macrophages cultured in the absence (control) or presence of diluted sealer extracts (1 : 4) 72 h after incubation. The cells were cultured in medium alone (M control) or with *Fusobacterium nucleatum* (F) or *Peptostreptococcus anaerobius* (P) preparations. Interferon-γ (I) was added where indicated. Moreover, * indicates a statistically significant difference between groups ($P < 0.05$). Black stars (★) indicate significant differences between control and stimulated groups, and white circles (○) indicate significant differences between macrophage sources ($P < 0.05$).

The M2 profile is involved in the progression of infectious diseases as well as in the healing process (Benoit *et al.* 2008, de Oliveira Mendes *et al.* 2010). Collagen synthesis and cell proliferation is associated with IL-10 production (Kawashima & Stashenko 1999). Interleukin-10 induces tissue homeostasis, which leads to the inhibition of pro-inflammatory cytokine production by activated T cells and macrophages (Kawashima *et al.* 1996, Stashenko *et al.* 1998, Gerber & Mosser 2001, Borish & Steinke 2003, de Brito *et al.* 2012). As expected, M2 macrophages produced higher levels of IL-10 than did M1 macrophages. FLPX sealer stimulated the production of IL-10 by M1 and M2 macrophages in *P. anaerobius*-stimulated cells cultured in the presence or absence of IFN-γ. Moreover, M2 macrophages that were pre-incubated with MTA extract and subsequently treated with *F. nucleatum* antigen in the absence of IFN-γ produced the highest levels of IL-10 ($P < 0.05$) (Fig. 4b). Previously, it was demonstrated that M2 macrophages treated with *F. nucleatum* in the absence of the sealers produced higher levels of IL-10 than did M1 macrophages under the same conditions (de Oliveira Mendes *et al.* 2010).

Finally, in previous studies (Rezende *et al.* 2005, 2007, de Oliveira Mendes *et al.* 2010), capillary tubes have been used as containers for the sealers because they allow for a similar pattern and a constant area of exposure of the peritoneal cells. In these circumstances, the low cellular viability (<50%) was observed in the presence of FLPX after 24 h of incubation (data not shown). This level of cellular viability did not allow at evaluating the effect of FLPX sealer on TNF and IL-12 production (24 h), and to assay for IL-10 and NO production (72 h). Hence, the cells were exposed to FLPX and MTA extracts as proposed by Bin *et al.* (2012). Interestingly, FLPX sealer extract was cytotoxic at the highest concentrations (1 : 1, 1 : 2) at 24 h and 72 h after stimulation. Moreover, in spite of it being demonstrated that the setting time does not significantly influence the cytotoxicity of the material (Balto 2004, de Oliveira Mendes *et al.* 2003), both materials were covered with aqueous solution of RPMI medium after setting. However, FLPX sealer is a newly released product in the market, and comprehensive clinical studies are necessary to compare the physiological response of this sealer to the *in vitro* results obtained in the present study.

Conclusions

This *in vitro* investigation showed that the association of root canal sealer with MTA did not improve the FLPX biological properties: FLPX impaired effector immune responses during inflammation (M1 macrophages), as well as during healing (M2 macrophages) responses.

Acknowledgements

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References

Balto HA (2004) Attachment and morphological behavior of human periodontal ligament fibroblasts to mineral trioxide aggregate: a scanning electron microscope study. *Journal of Endodontics* **30**, 25–9.

Bastos JCR, Alvarez JM, Marinho CR, Rizzo LV, Lima MR (2001) Macrophages from IL-12p40-deficient mice have a bias toward the M2 activation profile. *Journal of Leukocyte Biology* **71**, 271–8.

Benoit M, Desnues B, Mege JL (2008) Macrophage polarization in bacterial infections. *Journal of Immunology* **181**, 3733–9.

Bin CV, Valera MC, Camargo SEA *et al.* (2012) Cytotoxicity and genotoxicity of root canal sealers based on mineral trioxide aggregate. *Journal of Endodontics* **38**, 495–500.

Bogdan C, Vodovotz Y, Nathan C (1991) Macrophage deactivation by interleukin 10. *Journal of Experimental Medicine* **174**, 1549–55.

Borish LC, Steinke JW (2003) 2. Cytokines and chemokines. *Journal of Allergy and Clinical Immunology* **111**, 460–75.

Brackett MG, Marshall A, Lockwood PE *et al.* (2009) Inflammatory suppression by endodontic sealers after aging 12 weeks *In Vitro*. *Journal of Biomedical Materials Research Part B Applied Biomaterials* **91**, 839–44.

Brackett MG, Lewis JB, Messer RL, Lei L, Lockwood PE, Wataha JC (2011) Dysregulation of monocytic cytokine secretion by endodontic sealers. *Journal of Biomedical Materials Research Part B Applied Biomaterials* **97**, 49–57.

Bronte V, Zanovello P (2005) Regulation of immune responses by L-arginine metabolism. *Nature Reviews Immunology* **5**, 641–54.

Camargo CH, Camargo SE, Valera MC, Hiller KA, Schmalz G, Schweikl H (2009) The induction of cytotoxicity, oxidative stress, and genotoxicity by root canal sealers in mammalian cells. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics* **108**, 56–60.

de Brito LC, Teles FR, Teles RP, Totola AH, Vieira LQ, Sobrinho AP (2012) T-lymphocyte and cytokine expression in

The secretory activity of stimulated macrophages was investigated using this sealer concentration. After being phagocytised, microorganisms are killed via ROS and NO production (Garcia & Stein 2006, de Oliveira Mendes *et al.* 2010). Therefore, whether sealers would interfere with this response in the presence or absence of endodontic pathogens or zymosan (yeast positive control for ROS) was analysed.

Both macrophages subtypes produced significant amounts of ROS in response to zymosan, although M2 macrophages produced greater levels than M1 cells ($P < 0.05$). FLPX sealer inhibited the production of ROS by M1 and M2 macrophages. In contrast, Camargo *et al.* (2009) found that the ROS production was increased in response to other sealers such as Epiphany (Pentron Clinical Technologies, Wallingford, CT, USA), Acroseal (Septodont, Saint Maur des Fosses, France) and AH Plus (Dentsply De Trey, Konstanz, Germany). MTA did not affect ROS production by M2 macrophages. However, zymosan-stimulated M1 cells produced lower levels of ROS with MTA treatment compared to the control group. This interesting result was previously observed for M1 macrophages treated with EWT Pulp Canal sealer (de Oliveira Mendes *et al.* 2010), even though others have shown that MTA treatment does not affect ROS production (Rezende *et al.* 2007, Camargo *et al.* 2009).

The FLPX sealer decreased NO production by M1 and M2 macrophages in *F. nucleatum* and *P. anaerobius*-treated cells. Yoshino *et al.* (2013) reported low levels of NO in fibroblast culture supernatants after FLPX extracts were added. In contrast, NO production by bacteria-treated M1 and M2 macrophages increased when IFN-γ was added to the cultures, as described elsewhere (Rezende *et al.* 2007, de Oliveira Mendes *et al.* 2010). As described previously, there was no difference in NO production with MTA treatment when compared with the control group (Rezende *et al.* 2007). Taken together, these results show that FLPX sealer impairs the immune response against endodontic pathogens and decreases NO and ROS production.

Pro-inflammatory mediators, such as cytokines and microbial challenge, are responsible for the changes that occur in inflamed periapical tissues (Stashenko *et al.* 1998). Macrophages secrete a number of cytokines that perpetuate inflammation or direct healing processes (Sjogren *et al.* 1998, Brackett *et al.* 2011). Therefore, whether FLPX sealer interferes with TNF-α, IL-12 and IL-10 production by both macrophage subtypes was of interest. These cytokines are involved in the onset of the inflammatory processes (TNF-α), in the interconnection of the innate and adaptive immune responses (IL-12) or in immune regulation (IL-10). Heat-killed bacteria and IFN-γ stimuli were added to cell cultures to reproduce clinical conditions because anaerobic bacteria and other cytokines are frequently found in compromised periapical tissues (Rezende *et al.* 2005).

The major physiological functions of TNF-α are to induce neutrophil and macrophage migration to the site of the infection and activate these cells to kill microorganisms (Locksley *et al.* 2001, Xanthoulea *et al.* 2004); it also indirectly induces osteoclast activation (Stashenko 1990, Wang *et al.* 1997, Maciel *et al.* 2012). In contrast to a previous study, M2 macrophages produced higher levels of TNF-α than M1 macrophages (Rezende *et al.* 2005). FLPX sealer induced TFN-α production in *P. anaerobius*-stimulated M1 macrophages cultured in the presence of IFN-γ ($P \leq 0.05$). However, TNF-α production by *F. nucleatum*-stimulated M1 cells was not influenced by the presence of FLPX sealer. The opposite was observed in M2 cells: FLPX sealer significantly decreased TNF-α production in *F. nucleatum*-stimulated cells cultured in the presence or absence of IFN-γ. Other studies have also found that TNF-α production by macrophages was impaired by sealers (Perassi *et al.* 2004, Brackett *et al.* 2009, Sousa *et al.* 2009, de Oliveira Mendes *et al.* 2010). Moreover, MTA did not have negative effects on TNF-α production by M1 and M2 macrophages, as demonstrated by others (Rezende *et al.* 2005, 2007). These results suggest that in the absence of residual infection, MTA and FLPX sealers do not interfere with the production of TNF-α by either macrophage subtype.

Interleukin-12 is a pro-inflammatory cytokine that promotes cell-mediated immune responses in inflammatory and infectious disorders. Additionally, it functions as a link between the innate and the adaptive antigen-specific responses (Trinchieri & Scott 1995). As predicted, M2 did not produce IL-12p70 cytokine in this study. The gram- negative bacteria, *F. nucleatum*, stimulated M1 macrophages to produce higher levels of IL-12p70. FLPX sealer did not interfere with IL-12p70 production, although this sealer increased IL-12p70 production in *P. anaerobius*-stimulated cells in the presence of IFN-γ. Conversely, it was shown that a resin-based sealer (AH-Plus) decreased IL-12 production by LPS-stimulated monocytic cells (Brackett *et al.* 2011). Furthermore, MTA significantly stimulated IL-12p70 production by M1 macrophages in control cells and in *P. anaerobius*-stimulated cells, as demonstrated elsewhere (Rezende *et al.* 2005).

human inflammatory periapical lesions. *Journal of Endodontics* **35**, 481–5.

de Oliveira Mendes ST, Brito LCN, Rezende TMB *et al.* (2010) A decrease in innate immune response to infection in the presence of root canal sealers. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology* **109**, 315–23.

de Oliveira Mendes ST, Ribeiro Sobrinho AP, de Carvalho AT, de Souza Cortes MI, Vieira LQ (2003) *In vitro* evaluation of the cytotoxicity of two root canal sealers on macrophage activity. *Journal of Endodontics* **29**, 95–9.

Edwards JP, Zhang X, Frauwirth KA, Mosser DM (2006) Biochemical and functional characterization of three activated macrophage populations. *Journal of Leukocyte Biology* **80**, 1298–307.

Faria-Júnior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM (2013) Antibiofilm activity, pH and solubility of endodontic sealers. *International Endodontics Journal* **39**, 174–7.

Garcia X, Stein F (2006) Nitric oxide. *Seminars in Pediatric Infectious Diseases* **17**, 55–7.

Gerber JS, Mosser DM (2001) Reversing lipopolysaccharide toxicity by ligating the macrophage Fc gamma receptors. *Journal of Immunology* **166**, 6861–8.

Hasturk H, Kantarci A, Van Dyke TE (2012) Oral inflammatory diseases and systemic inflammation: role of the macrophage. *Frontiers in Immunology* **3**, 118.

Kawashima N, Stashenko P (1999) Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. *Archives of Oral Biology* **44**, 55–66.

Kawashima N, Kosaka T, Suda H (1996) Kinects of macrophages and lymphoid cells during development of experimentally induced periapical lesions in rat molars: a quantitative immunohistochemical study. *Journal of Endodontics* **22**, 311–26.

Kopitar-Jerala N (2006) The role of cystatins in cells of the immune system. *FEBS Letters* **580**, 6295–301.

Lee YY, Hung SL, Pai SF, Lee YH, Yang SF (2007) Eugenol suppressed the expression of lipopolysaccharide-induced proinflammatory mediators in human macrophages. *Journal of Endodontics* **33**, 698–702.

Locksley RM, Killeen N, Lenardo MJ (2001) The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell* **104**, 487–501.

Maciel ICF, Neves de Brito LC, Tavares WL *et al.* (2012) Cytokine expression in response to root canal infection in gnotobiotic mice. *International Endodontic Journal* **45**, 354–62.

Mahdi JG, Alkarrawi MA, Mahdi AJ, Bowen ID, Humam D (2006) Calcium salicylatemediated apoptosis in human HT-1080 fibrosarcoma cells. *Cell Proliferation* **39**, 249–60.

Mantovani A, Sozzani S, Locati M, Allavena P, Sica A (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends in Immunology* **23**, 549–55.

Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. *Journal of Immunology* **164**, 6166–73.

Mosser DM (2003) The many faces of macrophage activation. *Journal of Leukocyte Biology* **73**, 209–12.

Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. *Journal of Endodontics* **33**, 377–90.

Perassi FT, Filho IB, Berbert FL, Carlos IZ, de Toledo LR (2004) Secretion of tumor necrosis factor-alpha by mouse peritoneal macrophages in the presence of dental sealers, sealapex and endomethasone. *Journal of Endodontics* **30**, 534–7.

Rezende TM, Vargas DL, Cardoso FP, Sobrinho AP, Vieira LQ (2005) Effect of mineral trioxide aggregate on cytokine production by peritoneal macrophages. *International Endodontic Journal* **38**, 896–903.

Rezende TM, Vieira LQ, Cardoso FP, Oliveira RR, de Oliveira Mendes ST, Jorge ML (2007) The effect of mineral trioxide aggregate on phagocytic activity and production of reactive oxygen, nitrogen species and arginase activity by M1 and M2 macrophages. *International Endodontic Journal* **40**, 603–11.

Rezende TM, Vieira LQ, Sobrinho AP, Oliveira RR, Tanubman MA, Kawai T (2008) The influence of mineral trioxide aggregate on adaptive immune responses to endodontic pathogens in mice. *Journal of Endodontics* **34**, 1066–71.

Ribeiro Sobrinho AP, de Melo Maltos SM, Farias LM *et al.* (2001) Cytokine production in response to endodontic infection in germ-free mice. *Oral Microbiology Immunology* **17**, 344–53.

Salles LP, Gomes-Cornélio AL, Guimarães FC *et al.* (2012) Mineral trioxide aggregate-based endodontic sealer stimulates hydroxyapatite nucleation in human osteoblast-like cell culture. *Journal of Endodontics* **38**, 971–6.

Scarparo RK, Haddad D, Acasigua GAX (2010) Mineral trioxide aggregate based sealer: analysis of tissue reactions to a new endodontic material. *Journal of Endodontics* **36**, 1174–8.

Scelza MZ, Linhares AB, da Silva LE, Granjeiro JM, Alves GG (2011) A multiparametric assay to compare the cytotoxicity of endodontic sealers with primary human osteoblasts. *International Endodontic Journal* **45**, 112–8.

Silva EJNL, Rosa TP, Herrera DR, Jacinto RC, Gomes BPFA, Zaia AA (2013) Evaluation of cytotoxicity and physicochemical properties of calcium silicate-based endodontic sealer MTA fillapex. *Journal of Endodontics* **39**, 274–7.

Sjogren U, Ohlin A, Sundqvist G, Lemer UH (1998) Guttaperchastimulated mouse macrophages release factors that activate the bone resorptive system of mouse calvarial bone. *European Journal of Oral Sciences* **106**, 872–81.

Sousa LR, Cavalcanti BN, Marques MM (2009) Effect of laser phototherapy on the release of TNF-alpha and MMP-1 by endodontic sealer-stimulated macrophages. *Photomedicine and Laser Surgery* **27**, 37–42.

Stark LA, Din FV, Zwacka RM, Dunlop MG (2001) Aspirin-induced activation of the NFkappaB signaling pathway: a novel mechanism for aspirin-mediated apoptosis in colon cancer cells. *FASEB* **15**, 1273–5.

Stashenko P (1990) Role of immune cytokines in the pathogenesis of periapical lesions. *Endodontic and Dental Traumatology* **6**, 89–96.

Stashenko P, Teles R, D'Souza R (1998) Periapical inflammatory responses and their modulation. *Critical Reviews in Oral Biology and Medicine* **9**, 498–521.

Taylor PR, Martinez-Pomares L, Stacey M, Lin HH, Brown GD, Gordon S (2005) Macrophage receptors and immune recognition. *Annual Review of Immunology* **23**, 901–44.

Tarahinejad M, Hang CU, Lee SJ, Monsef M, Pitt Ford TR (1995) Investigation of mineral trioxide aggregate for root-end filling in dogs. *Journal of Endodontics* **21**, 603–8.

Tarahinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM (1998) Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. *Journal of Endodontics* **24**, 468–71.

Tarahinejad M, Chivian N (1999) Clinical applications of mineral trioxide aggregate. *Journal of Endodontics* **25**, 197–205.

Trinchieri G, Scott P (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions. *Research in Immunology* **146**, 423–31.

Trusk MA, Wilson ME, Dyke ICV (1978) The generation of chemiluminescence by phagocytic cells. In: Deluca MA, ed. *Methods in Enzymology*. London, UK: Academic Press, pp. 462–93.

van de Loosdrecht AA, Nennie E, Ossenkoppele GJ, Beelen RH, Langenhuijsen MM (1991) Cell mediated cytotoxicity against U 937 cells by human monocytes and macrophages in a modified colorimetric MTT assay. A methodological study. *Journal of Immunological Methods* **141**, 15–22.

Van Dyke TE (2008) The management of inflammation in periodontal disease. *Journal of Periodontology* **79**, 1601–8.

Verreck FA, De Boer T, Langenberg DM *et al.* (2004) Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco) bacteria. *Proceedings of the National Academy of Sciences* **101**, 4560–5.

Wang CY, Tani-Ishii N, Stashenko P (1997) Bone resorptive cytokine gene expression in periapical lesions in the rat. *Oral Microbiology and Immunology* **12**, 65–71.

Xanthoulea S, Pasparakis M, Kousteni S *et al.* (2004) Tumor necrosis factor (TNF) receptor shedding controls thresholds of innate immune activation that balance opposing TNF functions in infectious and inflammatory diseases. *Journal of Experimental Medicine* **200**, 367–76.

Yoshino P, Nishiyama CK, Modena KC, Santos CF, Sipert CR (2013) *In Vitro* Cytotoxicity of white MTA, MTA Fillapex® and portland cement on human periodontal ligament fibroblasts. *Brazilian Dental Journal* **24**, 111–6.

ANEXOS

ANEXO A

ATIVIDADES DESENVOLVIDAS DURANTE O CURSO DE DOUTORADO

Atuação profissional

Faculdade de Odontologia FEAD

2014 - atual Vinculo: Professor Adjunto, Enquadramento funcional:
Professor de Endodontia e clinica integrada. Carga horária: 12 horas/semanais.

Universidade Federal de Minas Gerais - Professor voluntário - FO-UFMG

2014 - 2016 Vínculo: Aluno de Doutorado, Enquadramento funcional:
Projeto de Extensão "Assistência Odontológica a Pacientes Transplantados da UFMG".Carga horária: 4/semanais.

Artigos completos publicados em periódicos:

- BRAGA JM; OLIVEIRA, RR ; MARTINS, RC ; VIEIRA, LQ ; SOBRINHO, AP RIBEIRO. Assessment of the cytotoxicity of a mineral trioxide aggregate-based sealer with respect to macrophage activity. Dent Traumatol. 2015 Oct;31(5):390-5. doi: 10.1111/edt.12190. Epub 2015 Jun 18.
- BRAGA JM; OLIVEIRA, RR ; MARTINS, RC; SOBRINHO, AP RIBEIRO. The effects of a mineral trioxide aggregate-based sealer on the production of reactive oxygen species, nitrogen species and cytokines by two macrophage subtypes. Int Endod J. 2014 Oct;47(10):909-19. doi: 10.1111/iej.12234. Epub 2014 Feb 3.

Artigos completos aceitos para publicação em periódicos:

- BRAGA-DINIZ, J. M.; SANTA-ROSA, C.C.; MARTINS, R. C.; SILVA, M. E. S. E.; VIEIRA, L. Q.; RIBEIRO-SOBRINHO, A. P. The need for endodontic treatment and systemic characteristics of hematopoietic stem cell transplantation patients.. BRAZILIAN ORAL RESEARCH. , 2017.

Resumos publicados em anais de congressos:

- Diniz JMB; OLIVEIRA, RR ; MARTINS, RC ; VIEIRA, LQ ; SOBRINHO, AP RIBEIRO . Efeito do Mineral trioxide Aggregate e do MTA Fillapex sobre a viabilidade, a aderência e a atividade fagocitária de macrófagos M1 e M2. In: 30ª Reunião anual da SBPqO, 2013, Águas de Lindóia. Brazilian Oral Reseach, 2013. v. 27. p. 31-31.

- BRAGA, Julia Mourão; Silva DC ; CORTES, M. I. S. ; BASTOS, J. V. . Pulpal Prognosis Luxated Permanent teeth. In: 17th world congress on dental traumatology, 2012, Rio de Janeiro. 17th word congress on dental traumatology, 2013.

- BAMBIRRA, B. H. S. ; BRAGA, J. M. ; MACIEL, K. F. ; ESPALADORI, M. C ; BRITO, L. C. N. ; VIEIRA, L. Q. ; RIBEIRO SOBRINHO, A. P. . . Efeito da associação MTA/Selênio sobre viabilidade celular, aderência e atividade fagocitária de macrófagos M1 e M2.. In: 32 reunião anual da SBPqO, 2015, Campinas/SP. Brasilian Oral Research 2015, 2015.

- ESPALADORI, M. C; MACIEL, K. F. ; BAMBIRRA, B. H. S. ; BRAGA, J. M. ; BRITO, L. C. N. ; VIEIRA, L. Q. ; RIBEIRO SOBRINHO, A. P. . Perfurações experimentais de furca em animais germ free tratadas com MTA acrescido de Selênio: Análise da Resposta imune. In: 32 reunião anual da SBPqO, 2015, Campinas/SP. Brasilian Oral Research 2015, 2015.

- SANTA-ROSA, C.C.; BRAGA, J.M.; SILVA, M. E. S. E.; VIEIRA, L. Q.; RIBEIRO-SOBRINHO, A. P.Estudo comparativo da necessidade de tratamento endodôntico de pacientes pré e pós-transplante de células tronco hematopoiéticas. In: XIII Encontro Científico da Faculdade de Odontologia da UFMG, 2016, Belo Horizonte.Revista Arquivos em Odontologia. , 2016.

- PARPINELLI, B. C.; SANTA-ROSA, C.C.; MACIEL, K. F.; ESPALADORI, M.C.; BRAGA, J.M.; BRITO, L. C. N.; VIEIRA, L. Q.; RIBEIRO-SOBRINHO, A. P. Modelo de avaliação das respostas imunológicas nas alterações pulpoperiapicais In: Semana do Conhecimento UFMG 2016 - XXV Semana de Iniciação Científica, 2016, Belo Horizonte. Anais Semana do Conhecimento UFMG 2016 - XXV Semana de Iniciação Científica. , 2016.

Apresentação de trabalho:

- Diniz JMB; OLIVEIRA, RR ; MARTINS, RC ; VIEIRA, LQ ; SOBRINHO, AP RIBEIRO. Efeito do Mineral Tioxide aggregate e do MTA Fillapex sobre a viabilidade, a aderência e a atividade fagocitária de macrófagos M1 e M2.. 2013. (Apresentação de Trabalho/Congresso 30ª Reunião anual da SBPqO).
- BRAGA, Julia Mourão; Silva DC ; CORTES, M. I. S. ; BASTOS, J. V. . Pulpal Prognosis Luxated permanent teeth. 2013. (Apresentação de Trabalho/Congresso).

Menção Honrosa:

- Diniz JMB; OLIVEIRA, RR ; MARTINS, RC ; VIEIRA, LQ ; SOBRINHO, AP RIBEIRO . Efeito do Mineral trioxide Aggregate e do MTA Fillapex sobre a viabilidade, a aderência e a atividade fagocitária de macrófagos M1 e M2. In: 30ª Reunião anual da SBPqO, 2013, Águas de Lindóia. Brazilian Oral Reseach, 2013. v. 27. p. 31-31.
- ESPALADORI, M. C; MACIEL, K. F. ; BAMBIRRA, B. H. S. ; BRAGA, J. M. ; BRITO, L. C. N. ; VIEIRA, L. Q. ; RIBEIRO SOBRINHO, A. P. . Perfurações experimentais de furca em animais germ free tratadas com MTA acrescido de Selênio: Análise da Resposta imune. In: 32 reunião anual da SBPqO, 2015, Campinas/SP. Brasilian Oral Research 2015, 2015.
- SANTA-ROSA, C.C.; BRAGA, J.M.; SILVA, M. E. S. E.; VIEIRA, L. Q.; RIBEIRO-SOBRINHO, A. P.Estudo comparativo da necessidade de tratamento endodôntico de pacientes pré e pós-transplante de células tronco hematopoiéticas. In: Encontro Científico da Faculdade de Itaúna, 2016.
- CRUZ I.R.D; BRAGA DINIZ, J.M. Revascularização Pulpar: Tratamento alternativo para dentes necrosados com rizogênese incompleta. In: II Encontro científico da Faculdade de Odontologia da FEAD.

Cursos e Aulas Ministradas

- Instrumentação Mecanizada em Endodontia de Dentes Decíduos. Faculdade de Odontologia FEAD. 4 horas/aula. Abril 2017
- Traumatismo Dentário. IES Pós-Graduação. 8 horas/aula. Agosto/2015.
- Traumatismo Dentário. IES Pós-Graduação. 8 horas/aula. Agosto/2014.

- Microscopia em Endodontia. Estação de Ensino Sociedade Educacional. 4horas/aula. Fevereiro/2014.
- Microscopia em Endodontia. Facsete. Faculdade de Sete Lagoas. 4horas/aula. 2014

Orientação de Trabalho de Conclusão de Curso:

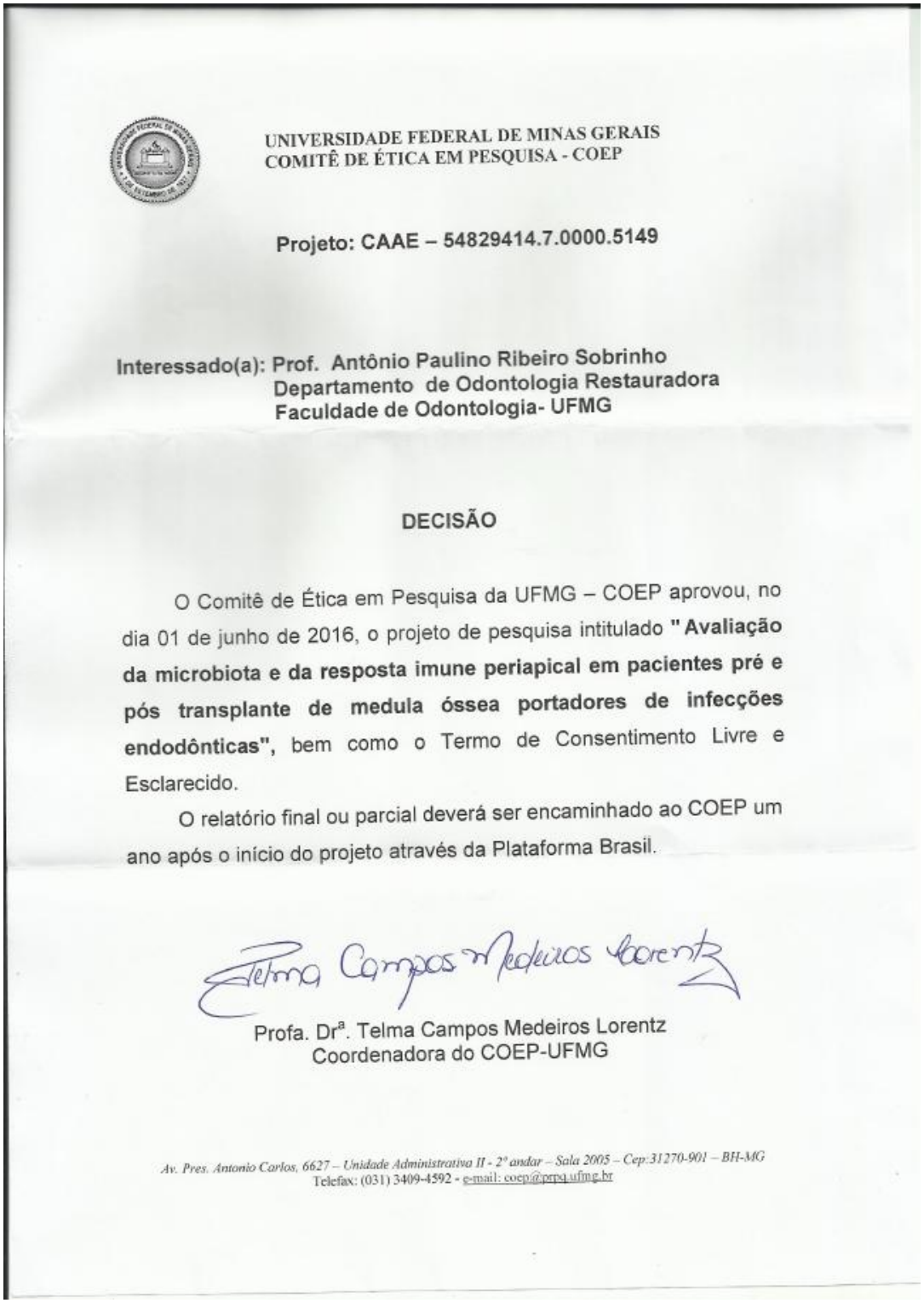
- Revascularização Pulpar: Tratamento alternativo para dentes necrosados com rizogênese incompleta. Aluna Izabelle Rayane Doralice da Cruz. Faculdade de Odontologia FEAD. 2017
- Medicações Intracanal na Revascularização Pulpar. Aluna: Ana Carolina Carvalho. Faculdade de Odontologia FEAD. 2017
- Uso do Agregado Trióxido Mineral no Tratamento de Dentes com Rizogênese incompleta: um relato de caso. Aluno: Jônatas Freire. Faculdade de Odontologia FEAD. 2017
- Revascularização pulpar em dentes permanentes jovens. Aluno: Victor Barcellos. Faculdade de Odontologia FEAD. 2016.
- Tratamento endodôntico em pacientes submetidos a radioterapia regiao cabeça e pescoço. Aluna: Francielle Silva. Faculdade de Odontologia FEAD. 2015.
- Agregado Tríoxido Mineral no Tratamento de Perfurações Radiculares e de Furca. Aluna: Vanessa Lacerda Guimarães. Faculdade de Odontologia FEAD. 2015.

Participação em Eventos:

- Congresso Internacional de Endodontia Canal 2016, 2016.
- Congresso Internacional de Endodontia Canal 2015, 2015.
- Congresso Internacional de Endodontia Canal 2014, 2014.

ANEXO B

PARECER COMITÊ DE ÉTICA



UNIVERSIDADE FEDERAL DE MINAS GERAIS
COMITÊ DE ÉTICA EM PESQUISA - COEP

Projeto: CAAE – 54829414.7.0000.5149

Interessado(a): Prof. Antônio Paulino Ribeiro Sobrinho
Departamento de Odontologia Restauradora
Faculdade de Odontologia- UFMG

DECISÃO

O Comitê de Ética em Pesquisa da UFMG – COEP aprovou, no dia 01 de junho de 2016, o projeto de pesquisa intitulado **" Avaliação da microbiota e da resposta imune periapical em pacientes pré e pós transplante de medula óssea portadores de infecções endodônticas",** bem como o Termo de Consentimento Livre e Esclarecido.

O relatório final ou parcial deverá ser encaminhado ao COEP um ano após o início do projeto através da Plataforma Brasil.

Telma Campos Medeiros Lorentz

Profa. Drª. Telma Campos Medeiros Lorentz
Coordenadora do COEP-UFMG

Av. Pres. Antonio Carlos, 6627 – Unidade Administrativa II - 2º andar – Sala 2005 – Cep:31270-901 – BH-MG
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UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DA MICROBIOTA E DA RESPOSTA IMUNE PERIAPICAL EM PACIENTES PRÉ E PÓS TRANSPLANTE DE MEDULA ÓSSEA PORTADORES DE INFECÇÕES ENDODÔNTICAS

Pesquisador: Antônio Paulino Ribeiro Sobrinho

Área Temática: Genética Humana:
(Haverá armazenamento de material biológico ou dados genéticos humanos no exterior e no País, quando de forma conveniada com instituições estrangeiras ou em instituições comerciais;);

Versão: 2

CAAE: 54829414.7.0000.5149

Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.569.493

Apresentação do Projeto:

Estudo experimental, que avaliará a presença de espécies microbianas utilizando-se a técnica do seqüenciamento de DNA em 20 pacientes portadores de dentes com necrose pulpar que foram submetidos ao Transplante de Células Tronco Hematopoiéticas (TCTH) e 20 que serão submetidos ao TCTH. As amostras coletadas em pacientes atendidos na Clinica de TMO da Faculdade de Odontologia da UFMG quanto ao seu conteúdo microbiano e ao perfil de citocinas expressas nos tecidos periapicais adjacentes. O prontuário odontológico e os exames médicos complementares serão utilizados para uma avaliação completa do perfil dos participantes.

Segundo o pesquisador, a prevalência do caráter polimicrobiano das infecções endodônticas tem sido estudada ao longo dos anos. O desenvolvimento de metodologia filogenética que analisa e sequencia o gene 16S rRNA têm sido um dos métodos moleculares utilizados quando se quer examinar e caracterizar a diversidade de uma comunidade microbiana, tendo sido utilizada na caracterização da população microbiana de pacientes saudáveis, pacientes com doença periodontal e pacientes com infecções endodônticas. O TCTH envolve a ablação das células anormais ou malignas com altas doses de quimioterapia com ou sem radioterapia corpórea total, o

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que leva o paciente a um estado de imunossupressão. Essa condição permite que ele fique mais susceptível a infecções que podem provocar uma considerável morbidade ou mesmo levar o paciente ao óbito. Utilizando a técnica de amplificação e seqüenciamento do gene 16S rRNA, procurará correlacionar a microbiota presente em infecções endodônticas e os mecanismos imunológicos de defesa e destruição dos tecidos periapicais, dos pacientes na fase de pré e pós transplante de Células Tronco Hematopoiética.

Na metodologia descreve-se: ANÁLISE MICROBIOLÓGICA:A coleta dos espécimes clínicos para identificação microbiana será realizada da seguinte forma:O dente selecionado, após os procedimentos clínicos iniciais, terá sua coroa clínica completamente isolada. A coroa será desinfectada e uma lima apropriada será inserida em um dos canais radiculares,e depois cortar-se-á 4 milímetros de sua parte ativa, inserindo-a em uma solução apropriada para ser armazenada até o seu processamento.ETAPA LABORATORIAL: o DNA será quantificado e depois será feito seu sequenciamento, de acordo com protocolo, e analisado os dados. ANÁLISE IMUNOLÓGICA:depois de finalizada a instrumentação dos SCR, um cone absorvente será inserido em seu interior, ultrapassando o forame radicular em um milímetro, para que o mesmo entre em contato com os tecidos perirradiculares, sendo mantido por 2 minutos e, posteriormente, será inserido em um eppendorf. O mesmo procedimento será realizado após a remoção da medicação intra-canal utilizada. A quantificação das citocinas IL-1, IL-10, IL-12, IL-23, IL-17 IFN-, TNF-, RANKL e IL-4, será realizada pelo Real Time PCR, amplificando-se o mRNA e quantificando-se o cDNA provenientes das amostras clínicas.

Os participantes realizarão radiografias, definição do diagnóstico e receberão tratamento de canal, a ser realizado na FO/UFMG, situada na cidade de Belo Horizonte MG, obedecendo às normas da instituição. Após a coleta, inserindo-se uma lima endodôntica e cone absorvente no interior dos canais radiculares, as amostras serão levadas ao Laboratório de Microbiologia para serem processadas.

Objetivo da Pesquisa:

São definidos no projeto:

Objetivo Primário:Avaliar a microbiota de Sistemas de Canais Radiculares humanos infectados dos indivíduos pré e pós transplante de células tronco hematopoiéticas, antes e após o preparo mecânico químico, correlacionando tais achados ao perfil de citocinas presentes nos tecidos (as alterações) perirradiculares adjacentes.

Objetivo Secundário:• Identificar através da técnica de seqüenciamento de nova geração do 16S rRNA, a diversidade microbiana do SCRs dos indivíduos que serão submetidos ao transplante de

células tronco hematopoiéticas (fase pré transplante).• Identificar através da técnica de seqüenciamento de nova geração do 16S rRNA, a diversidade microbiana do SCRs dos indivíduos que foram submetidos ao transplante de células tronco hematopoiéticas(fase pós transplante).• Identificar e quantificar a expressão de citocinas nas lesões perirradiculares logo após a instrumentação do SCR dos indivíduos que serão submetidos ao transplante de células tronco hematopoiéticas (fase pré transplante).• Identificar e quantificar a expressão de citocinas nas lesões perirradiculares uma semana após a instrumentação do SCR dos indivíduos que serão submetidos ao transplante de células tronco hematopoiéticas (fase pré transplante).• Identificar e quantificar a expressão de citocinas nas lesões perirradiculares logo após a instrumentação do SCR dos indivíduos que foram submetidos ao transplante de células tronco hematopoiéticas (fase pós transplante).• Identificar e quantificar a expressão de citocinas nas lesões perirradiculares uma semana após a instrumentação do SCR dos indivíduos que foram submetidos ao transplante de células tronco hematopoiéticas (fase pós tranplante).• Correlacionar os dados microbiológicos e imunológicos.

Avaliação dos Riscos e Benefícios:

São relatados no projeto:

Riscos: Os riscos são inerentes ao tratamento endodôntico.

Benefícios:As informações obtidas resultarão no melhor conhecimento dos fenômenos inflamatórios que ocorrem nospacientes portadores de infecção endodôntica na fase pré e pós transplante de medula óssea.

Comentários e Considerações sobre a Pesquisa:

Projeto relevante para a área da saúde.

Considerações sobre os Termos de apresentação obrigatória:

- Folha de rosto preenchida e assinada em 01/04/16.
- Projeto aprovado pelo Programa de Pós-Graduação em Odontologia na FO/UFMG (CPGO) em 24/02/15.
- Parecer aprovado no Departamento de Clínica, Patologia e Cirurgia Odontológica da FO/UFMG em 24/02/15.
- Parecer aprovado do Departamento de Odontologia Restauradora da FO/UFMG, em 21/03/16.
- TCLE apresentado como carta convite, assegurando a voluntariedade, o anonimato, e a desistência a qualquer momento do projeto, sem qualquer prejuízo. Informou os contatos dos pesquisadores responsáveis para dúvidas e do COEP. Campo de assinaturas presentes. Descreveu

a metodologia, os benefícios e riscos.

As diligências corrigidas e conferidas, foram relatadas em resposta ao COEP:

No item Participação e compensação foi acrescentado as seguintes frases: "Pacientes em tratamento no Programa de assistência odontológica a pacientes transplantados da UFMG são constantemente avaliados em relação ao seu estado de saúde global antes de qualquer intervenção odontológica, minimizando a ocorrência complicações pós operatórias. Entretanto, caso você sinta algum incomodo após o tratamento você poderá entrar em contato com a Dra Julia Mourão Braga Diniz no telefone 33357629 sem nenhum constrangimento.

No item Critérios de seleção no 3º parágrafo agora lê-se : "Qualquer dúvida referente à sua participação e às questões éticas dessa pequisa poderá ser esclarecida a qualquer momento no COEP. No item autorização, na quinta linha, novamente foi enfatizado que o participante poderá entrar em contato com o COEP na frase onde se lê : " Caso eu tenha alguma dúvida em relação às questões éticas desse trabalho poderei entrar em contato com o COEP."

No item Autorização o termo "cópia" foi substituído pelo termo via como sugerido no parecer. A alteração se encontra na sexta linha do item autorização.No item confidencialidade dos dados na 4ª linha foi acrescentado a frase " Os dados serão armazenados por no mínimo 5 anos e você pode consultá-los a qualquer momento"

Recomendações:

Segundo a Resolução CNS 466/12:

1- Informar os correios eletrônicos das pesquisadores envolvidas no TCLE.

2- Assegurar que os 20 pacientes envolvidos no projeto são adultos, acima dos 18 anos, uma vez que não há TALE, nem especificação quanto à idade no projeto.

Este Comitê confia que as mudanças serão realizadas pelos pesquisadores.

Conclusões ou Pendências e Lista de Inadequações:

Sou, S.M.J., favorável à aprovação do projeto.

Tendo em vista a legislação vigente (Resolução CNS 466/12), o COEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de

notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

O presente projeto, seguiu nesta data para análise da CONEP e só tem o seu início autorizado após a aprovação pela mesma.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_401100.pdf	25/05/2016 17:08:53		Aceito
Outros	crespostacoep.doc	25/05/2016 17:08:27	Antônio Paulino Ribeiro Sobrinho	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	Termoconsentimento.doc	25/05/2016 17:05:34	Antônio Paulino Ribeiro Sobrinho	Aceito
Folha de Rosto	rosto.pdf	05/04/2016 08:14:52	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	aprovadep.pdf	22/03/2016 21:48:28	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	parecer2.pdf	22/02/2016 12:27:01	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	parecer1.pdf	22/02/2016 12:26:17	Antônio Paulino Ribeiro Sobrinho	Aceito
Outros	parecer.pdf	22/02/2016 12:25:15	Antônio Paulino Ribeiro Sobrinho	Aceito
Projeto Detalhado / Brochura Investigador	Elementos pre textuais TMO.doc	09/11/2014 20:59:11		Aceito
Projeto Detalhado / Brochura Investigador	ProjetoDoutorado Julia.doc	09/11/2014 20:58:20		Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Sim

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

Continuação do Parecer: 1.569.493

BELO HORIZONTE, 02 de Junho de 2016

Telma Campos Medeiros Lorentz

Assinado por:
Telma Campos Medeiros Lorentz
(Coordenador)

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