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EXPRESSÃO DE MATRIZ DE METALOPROTEINASE-2 CORRELACIONADA A BACTÉRIAS GRAM-NEGATIVAS EM LESÕES PERIAPICAIS SINTOMÁTICAS E ASSINTOMÁTICAS

Curitiba
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Dissertação apresentada ao Programa de Pós-Graduação em odontologia da Pontifícia Universidade Católica do Paraná, como parte dos requisitos para obtenção do título de Mestre em odontologia, Área de Concentração em Endodontia.

Orientador : Everdan Carneiro

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Curitiba, 31 de outubro de 2014.

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Expressão de matriz de metaloproteinase-2 correlacionada a bactérias gram-negativas em lesões periapicais sintomáticas e assintomáticas.

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Resumo

Introdução: Determinar se a hipótese que a expressão de matriz metaloproteinase-2 (MMP-2) é significativamente elevada em pacientes com periodontite apical sintomática e correlacionar com a quantidade detectada de bactérias gram-negativas. Métodos: Vinte pacientes com lesões periapicais foram divididos em 2 grupos: o grupo sintomático (ASSIN) incluindo 10 pacientes expressando lesão periapical e dor, e o grupo assintomático (SIN), incluindo 10 pacientes sem expressar dor. Todos os dentes foram retratados endodonticamente, pelo menos, dois anos antes da cirurgia periapical. As lesões periapicais foram coletadas e serialmente seccionadas em cortes de 4µm. Pelo menos duas secções foram processadas para exame microscópico com hematoxilina-eosina. Outras secções foram processadas para imunohistoquímica. Para a MMP-2, a área selecionada com células imunopositivas foi medida, e a percentagem de MMP-2, em relação a área total do campo microscópico foi calculada. Para as bactérias gram-negativas, o número de bactéria que apresentavam a cor rosa vermelhada foi contado por campo microscópico. O teste T-Student foi utilizado para comparar os grupos SIN e ASSIN. O Coeficiente de correlação de Pearson foi utilizado para determinar uma correlação significativa entre o número de bactérias gram-negativas e os níveis de MMP-2. O nível de significância foi estabelecida em $p < 0,05$. Resultados: Pelo percentual de área do MMP-2, o grupo ASSIN mostrou uma média estatisticamente maior do que o grupo SIN ($p = 0,027$). O grupo SIN mostrou um aumento estatisticamente significativo no número de células gram-negativas ($p = 0,003$) quando comparado ao ASSIN. Não houve correlação positiva estatisticamente significativa entre o número de bactéria gram-negativas e a porcentagem de área de MMP-2 ($p = 0,187$). Conclusão: Existe fraca evidência de um papel significativo de bactérias gram-negativas e MMP-2 em lesões periapicais sintomáticas.

Palavras-chave: bactérias gram-negativas; matriz metaloproteinase-2; lesões periapicais; sintomático.

Introdução

A periodontite apical é uma doença inflamatória dos tecidos peri-radiculares causadas por infecção microbiana persistente dentro do sistema de canal radicular do dente afetado.[1] Pode ter uma fase aguda ou crônica. A inflamação aguda (sintomática) é uma resposta exsudativa, enquanto que a inflamação crônica é mais proliferativa (assintomática).[2]

As metaloproteinases da matriz (MMPs) são uma família de endopeptidases metais-dependentes que representam a maioria das classes de enzimas responsáveis pela degradação dos componentes da matriz extracelular (ECM).[3] A MMP-1 tem sido relacionada como chave na iniciação da fase de reabsorção óssea na periodontite apical.[4] Além de MMP-2, MMP-3, MMP-8, MMP-9 e MMP-13 que foram descritas em lesões periapicais[5,6,7,8,9] e desempenham um papel de suma importância durante o desenvolvimento de patologia periapical.[8,10] Entretanto, foi sugerido recentemente que há boas evidências para suspeitar de um papel significativo de bactérias gram-negativas e MMP-9 em lesões periapicais sintomáticas.[11] A MMP-2 também conhecida como gelatinase A, é uma proteína ligada à membrana que é de suma importância para o *turnover* da matriz extracelular, preferencialmente clivar colágeno tipo IV, V, VII e XI e gelatina.[12] A imunomarcação para a MMP-2 na lesão periapical foi expressa, principalmente pelos fibroblastos, células endoteliais, monócitos / macrófagos e osteoblastos.[5] Durante a fase de desenvolvimento da lesão, a MMP-2 e MMP-9 foram descritas, por desempenhar um papel crítico.[10] Por outro lado, tem sido relatado que os níveis de MMP-9 aumentaram, enquanto que os níveis de MMP-2 diminuíram em comparação com polpas inflamadas e polpas saudáveis.[13] Além disso, foram também relatadas diferentes níveis entre MMP-2 e MMP-9 em abscessos periapicais agudos e crônicos.[14]

O objetivo deste estudo foi testar a hipótese de que a expressão da MMP-2 é significativamente elevada em pacientes com periodontite apical sintomática e correlacionar com a quantidade detectada de bactérias gram-negativas.

Materiais e Métodos

Seleção dos Pacientes

Vinte pacientes com lesões periapicais foram selecionados para este estudo. O diagnóstico de periodontite apical foi baseado principalmente no exame radiográfico, o que demonstra a perda óssea clara e desaparecimento do espaço do ligamento periodontal na região periapical. Todas as amostras de tecido (n = 20) foram da região anterior superior envolvendo apenas um dente de cada paciente. A idade dos pacientes variou de 19 a 41 anos. Apenas pacientes livres de medicação foram incluídos na pesquisa. Todos os procedimentos foram realizados após a obtenção da aprovação do comitê de Ética da Pontifícia Universidade Católica do Paraná, Brasil, Número Parecer: 459.509, e os pacientes assinaram um formulário com o termo de consentimento. Os pacientes foram divididos em dois grupos: o grupo sintomático (SIN), que incluía 10 pacientes com queixa de dor severa e constante, antes da cirurgia endodôntica, e o grupo assintomático (ASSIN), que incluía 10 pacientes sem dor ou sintomas. Todos os dentes foram retratados endodonticamente em um período de até dois anos antes da cirurgia periapical. No grupo assintomático, a imagem das lesões periapicais tinha aumentado quando comparado com a radiografia inicial do retratamento endodontico. No grupo SIN a periodontite aguda estava presente e poderia sugerir insucesso no retratamento. A cirurgia periapical foi realizada em todos os dentes e o tecido periapical foi coletado e imediatamente imersos em formalina neutra 10% tamponada durante 24 horas e, em seguida, cortados em secções de 4µm. Duas secções foram processadas para exame imuno-histoquímico. Outras duas secções foram processadas para exame histológico utilizando coloração de gram-negativo.

Imunohistoquímica

As secções de tecido em lâmina de vidro foram desparafinadas por aquecimento a 60 ° C, seguido de passagens através de xilol e graduação alcoólica e finalmente, a água. A recuperação antígênica foi efetuada por fervura das lâminas em solução tampão de citrato 10MN, pH 6,0 durante 10 minutos, seguido de

esfriamento no mesmo tampão durante 30 min. A peroxidase endógena foi bloqueada por incubação com 0,3% de H2O2 em metanol durante 15 min. Logo em seguida, os cortes foram incubados em soro normal 3%, diluído em água destilada à temperatura ambiente durante 20 min., e foram incubadas sequencialmente com MMP-2 (37150, ABCAM,EUA). Após o período de incubação, (overnight), as secções foram lavadas com PBS e incubadas com HRP WITHADVANCETM Enzima (DAKO®, EUA). As secções foram lavadas com TBS tris pH 7,3. Os cortes foram tratados com substrato 3, 3 '-diaminobenzidina tetrahidrocloreto (DAB) (SIGMA ALDRICH, D5637, ST. LOUIS, MO, EUA) para um máximo de 5 minutos à temperatura ambiente. O DAB foi enxaguado, posteriormente, o contraste com hematoxilina Mayer e coberturas deslizantes foram realizadas conforme as etapas finais antes das lâminas serem analisadas em microscópio ótico.

Coloração Gram

Após desparanifização e reidratação, os cortes foram corados em violeta cristal 1% por 15 minutos. Os cortes foram então inundados por 30 segundos com Lugol iodo por acetona por 2 segundos. As secções foram lavadas com água imediatamente após e contrastadas com carbol-fucsina durante 20 segundos e, em seguida, lavado com água e secas com papel absorvente. Bactérias gram-negativas foram coradas com pigmento rosa-vermelho.[11]

Análise Quantitativa de Imagens

Para cada secção positiva, dez campos microscópicos mostrando a maior imuno-positividade foram selecionados e fotomicrografadas com uma ampliação de 400X (BX50, Olympus, Tóquio, Japão). Todas as etapas de análise imunohistoquímica e gram foram realizadas utilizando um software de análise de imagem (Dino-eye, Anmo Eletrocnics Corporation, Taiwan). Para MMP-2, a fração de área das células positivas foi medida, e a percentagem de MMP-2 imunopositiva em relação à área total do campo do microscópio foi calculada. Para as células gram-negativas coradas, o número de células que apresentam a cor rosa-vermelho foi contado por campo microscópico.[11]

Análise Estatística

Os dados foram analisados por meio de testes de normalidade Kolmogorov-Smirnov e Shapiro-Wilk. Os testes mostraram que os dados apresentaram distribuição normal (paramétrico). Foi utilizado o teste T Student para comparar os grupos SIN e ASSIN. O Coeficiente de correlação de Pearson foi utilizado para determinar uma correlação significativa entre o número de células e o nível de MMP-2. O nível de significância foi fixado em p≤.05. A análise estatística foi realizada.

Resultados

Para a MMP-2, a percentagem de área imunopositiva, o grupo ASSIN apresentou uma percentagem significativamente maior do que a área do grupo SIN (p = 0,027, Figura 1). Para as bactérias gram-negativas, o grupo SIN mostrou um número significativamente maior do que a média do grupo assintomático. (p = 0,003, Figura 2).

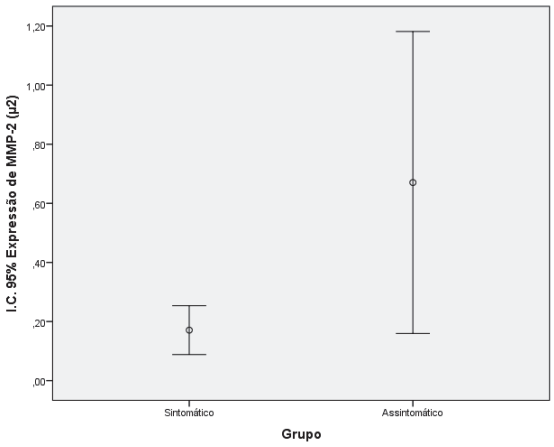

Figura 1 – Quantidade de bactérias Gram-Negativa

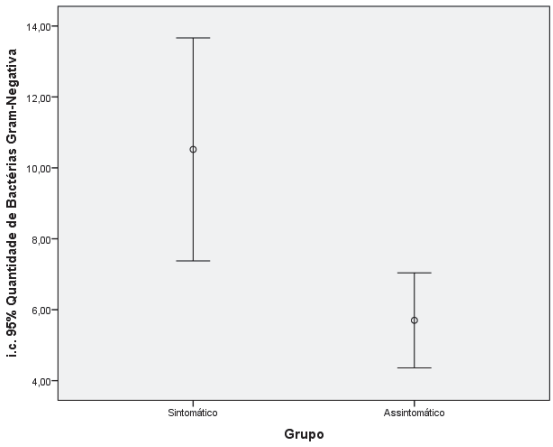

Figura 2 – Expressão de MMP-2

A expressão imunohistoquimica de MMP-2 foi expressa, principalmente por macrófagos, células do tipo fibroblasto, células epiteliais, células endonteliais (Figura 3). Não houve uma correlação positiva estatisticamente significante entre o número de bactérias gram-negativas e da porcentagem de área de MMP-2 (p = 0,187).

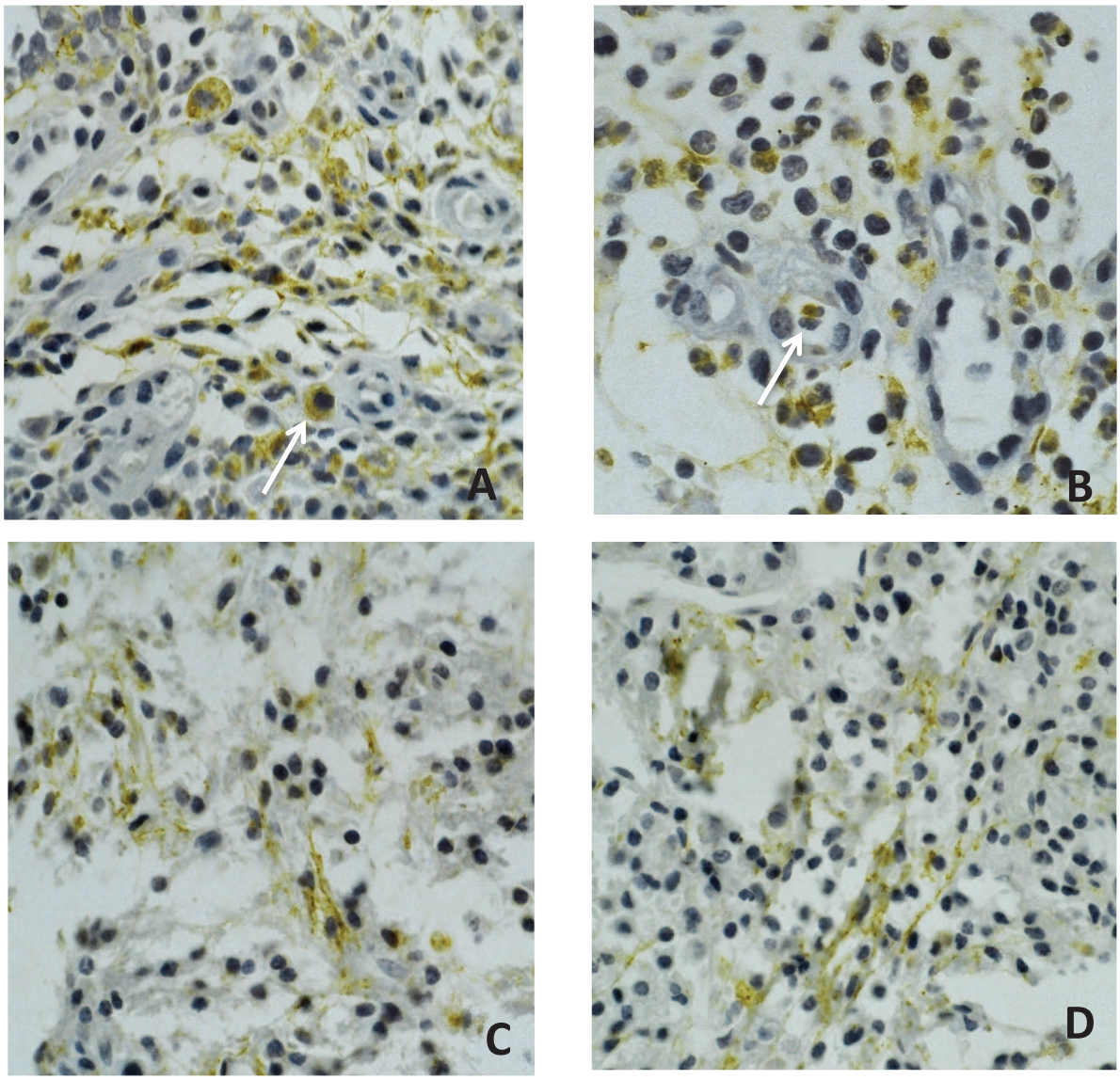

Figura 3 - A: Grupo Assintomático – Área com células inflamatórias imunopositivas para MMP-2, na seta sugestivo de macrófago. B: Grupo Sintomático - Área com células inflamatórias imunopositivas para MMP-2, na seta sugestivo de neutrófilo. C: Grupo Assintomático – Área com células imunopositivas para MMP-2. D: Grupo Assintomático – Área com células imunopositivas para MMP

Discussão

As MMPs têm sido identificadas tanto em inflamações pulpares quanto periapicais.[13,6] As MMPs podem também estar envolvidas em processos fisiológicos quanto patológicos, fenômenos que ocorrem no meio bucal.[15]

As MMPs desenvolvem um importante papel na destruição do tecido pulpar e tem sido proposto que a MMP-1, MMP-8, MMP-9 e MMP-13 juntas estão envolvidas em casos de expansões de lesões císticas.[7,8,16]

Estudos anteriores já mostraram imunomarcação para MMP-2.[5,10] No presente estudo, marcações positivas para MMP-2 foram detectadas em células semelhantes a macrófagos, células semelhantes a fibroblastos, células epiteliais e endoteliais.

O aumento no nível de MMP-2 foi observado em cultura de células de polpa e ligamento periodontal humanos, quando estas foram expostas a *porphyromonas endodontalis and gingivalis*, sendo comumente estas bactérias associadas a abcessos periapicais agudos. Por outro lado, a MMP-2 pode ser detectada em amostras de abcessos periapicais agudos a níveis menos expressivos quando comparados com a MMP-9.[14]

Diferentes padrões na expressão entre as gelatinases MMP-2 e MMP-9 tem sido descritos na literatura. Estudos em cultura de células de polpa humana demonstraram produzir primeiramente MMP-2 e MMP-9, entretanto, quando a IL-1 foi introduzida no meio, as células tiveram os níveis de MMP-2 elevados, contudo IL-1 não afetou os níveis de MMP-9 no oitavo dia do período de cultura.[17]

O achado mais interessante no presente estudo foi o padrão de expressão de MMP-2, o qual foi o oposto de um estudo usando a mesma metodologia, mas estudando a MMP-9.[11] MMP-2 e MMP-9 tem sido descrito por representar um importante papel durante o desenvolvimento da lesão periapical.[10] Por outro lado, quando a lesão periapical é estabelecida, as gelatinas podem apresentar um diferente comportamento. Nesse estudo, a marcação de MMP-2 no grupo assintomático foi mais alta quando comparado com o grupo sintomático, contudo, isto pode sugerir um diferente papel quando comparado com a MMP-9 em periodontite aguda.

Tem sido sugerido que algumas bactérias gram negativas estão envolvida em lesões periapicais sintomáticas.[18] Contudo no presente estudo não houve correlação entre bactérias gram negativas e o aumento da atividade de MMP-2 em lesões periapicais sintomáticas. Isto pode sugerir um equilíbrio entre as gelatinases, provavelmente existem diferentes padrões, quando a MMP-9 aumenta sua expressão, a MMP-2 recebe sinais para diminuir.

Para bactérias gram negativas, o grupo SIN apresentou estatisticamente maior número do que o grupo ASSIM. Baseado nesses resultados pode-se sugerir que as bactérias gram negativas podem não desempenhar um importante papel na atividade de MMP-2 quando a lesão está asintomática. Com base nos resultados, pode-se concluir que as bactérias gram-negativas não podem desempenhar um papel importante na atividade de MMP-2, quando a lesão periapical é sintomática.

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ARTIGO EM INGLÊS

Expression levels of matrix metalloproteinase-2 and gram-negative bacteria in symptomatic and asymptomatic periapical lesions

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Abstract

Introduction: To determine whether the hypothesis that the expression of matrix metalloproteinase (MMP-2) is significantly elevated in patients with symptomatic apical periodontitis and to correlate this with the detected amount of gram-negative bacteria. Methods: Twenty patients with periapical lesions were divided into 2 groups: ten symptomatic (SYM) group included 10 patients expressing pain with periapical lesion, and the asymptomatic (ASYM) group included 10 patients expressing no pain. All teeth had been endodontically retreated at least two year before the periapical surgery. The periapical lesions were collected. Periapical lesions were serially cut into 4μm sections. At least 2 sections were processed for histological examination using hematoxylin-eosin stain. Other sections were processed for immunohistochemical examination. For MMP-2, the area fraction of the positive cells was measured, and the percentage of the MMP-2 imunopositive area to the total area of the microscopic field was calculated. For gram-negative stain cells, the number of cells showing the pink-red color was counted per microscopic field. The Student's test was used to compare SYM and ASYM groups. The Pearson correlation coefficient was used to determine a significant correlation between the number of cells and MMP-2 levels. The significance was set at $p<.05$. **Results:** For MMP-2 area percent the ASYM group showed statistically significant higher mean than SYM group ($p=.027$).

The SYM group showed a statistically significant higher mean number of gram-negative cells ($p=.003$). There was no statistically significant positive correlation between the number of gram-negative cells and the MMP-2 area percent ($p=.187$). Conclusion**:** There is weak evidence to suspect a significant role of gram-negative bacteria and MMP-2 in symptomatic periapical lesions.

Keys words: gram-negative bacteria; matrix metalloproteinase-2; periapical lesions; symptomatic

Introduction

Apical periodontitis is an inflammatory disorder of periradicular tissues caused by persistent microbial infection within the root canal system of the affected tooth.[1] The apical periodontitis can be an acute or chronic stage. The acute inflammation (symptomatic) is an exudative response, whereas chronic inflammation is more proliferative (asymptomatic).[2]

Matrix metalloproteinases (MMPs) are an important family of metal-dependent endopeptidases that represent the major class of enzymes responsible for degradation of extracelular matrix (ECM) components.[3] MMP-1 has been implicated as a key in the initiation of the bone resorptive phase of apical periodontitis.[4] In addition MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 have been described in periapical periodontitis[5,6,7,8,9] and plays an important role during periapical pathology development.[8,10] However, it has been recently suggested that there is good evidence to suspect a significant role of gram-negative bacteria and MMP-9 in symptomatic periapical lesions.[11]

MMP-2 also known as gelatinase A, is a membrane-bound protein that is important for extracelular matrix turnover, preferentially cleaving collagen types IV, V, VII and XI and gelatina.[12] Immunoreactivity to MMP-2 in the periapical lesion was mainly expressed by fibroblasts, keratinocytos, endotelial cells, monocytes/macrophages and osteoblasts.[5] During the phase of the lesion development, MMP-2 and MMP-9 have been described that can play a critical role.[10] On the other hand, it has been reported that MMP-9 levels increased, whereas MMP-2 levels decreased in inflamed pulps compared with healthy pulps.[13] Moreover, it also been reported different levels between MMP-2 and MMP-9 in acute and chronic periapical abscesses.[14]

The aim of this study was to test the hypothesis that the expression of MMP-2 is significantly elevated in patients with symptomatic apical periodontitis and to correlate this to the detected amount of gram-negative bacteria.

Material and Methods

Patient Selection

Twenty patients, with noncontributory medical histories and diagnosed with periapical lesions were selected for this study. The diagnosis of apical periodontitis was based mainly on radiographic examination, demonstrating clear bone loss and disappearance of the periodontal ligament space in the periapical region. All tissue samples (*n*=20) were from upper anterior area involving one tooth only of each patient. The age of the patients ranged from 19 to 41 years old. Only medically free patients were included in this research. All procedures were performed after obtaining proper institucional review board approval based on the regulations of the Ethical Commiteee of the Pontifical Catholic University of Parana, Brazil, and patients signed an informed consent form. The patients were divided into two groups: the symptomatic (SYM) group, which included 10 patients complaining from severe pain and fullness before endodontic surgery, and the asymptomatic (ASYM) group, which included 10 patients with no pain or symptoms.

All teeth had been endodontically retreated at least two years before the periapical surgery. In the ASYM group, the image of the periapical lesions had increased when it compared with the initial radiographic of endodontics retreatment. In the SYM group the acute periodontitis was present and it could suggest failure in the retreatment.

The periapical surgery was performed in all teeth and the periapical tissue were collected and immediately immersed in 10% neutral buffered formalina for 24 hours and then serially cut into 4-µm sections. At least two sections were processed for immunohistochemical examination. Other sections were processed for histologic examination using gram-negative stain.[11]

Immunohistochemistry

The tissue sections on glass slide were deparaffinized by heating at 60 °C, followed by passages through xylene and alcohol grades, and ultimately to water. Antigen retrieval was performed by boiling the slides in 10 MN citrate buffer, pH 6.0

for 10 minutes (min), followed by cooling in the same buffer for 30 min. The endogenous peroxidase was quenched by incubating slides with 0.3% H_2O_2 in methanol for 15 min. Soon afterwards, the sections were incubated in 3% normal serum diluted in distilled water at room temperature for 20 min, and were sequentially incubated with MMP-2 (37150, Abcam, USA). Following the incubation period, (overnight), the sections were washed with PBS and incubated with AdvanceTM HRP Enzyme (DAKO®, USA). The sections were washed with TBS tris pH 7.3. The sections were treated with substrate 3, 3′-diaminobenzidine tetrahydrochloride (DAB) (Sigma Aldrich, D5637, St. Louis, MO, USA) for up to 5 min at room temperature. DAB was rinsed. Afterward, counterstaining with Mayer hematoxylin and cover slipping were performed as the final steps before slides were examined under a light microscope.

Gram Stain

After deparaffinizattion and rehydratation, sections were stained in 1% Crystal violet for 15 minutes. Sections were then flooded for 30 seconds with lugol iodine by acetone for 2 seconds. The sections were washed immediately after with water and counterstained with carbolfuchsin for 20 seconds and then washed with water and blotted dry. Gram-negative bactéria were stained pink-red.[11]

Quantitative Image Analysis

For each positive section, 10 microscopic fields showing the highest immunopositivity were selected and photomicrographed at a magnification of 400X (BX50; Olympus, Tokyo, Japan). All steps for immunohistochemical and gram stain analysis were performed using image analysis software (Dino-eye, Anmo electronics corporation, Taiwan). For MMP-2, the área fraction of the positive cells was measured, and the percentage of the MMP-2- immunopositive area to the total área of the microscope field was calculated. For gram-negative-stained cells, the number of cells showing the pnk-red color was counted per microscopic field.[11]

Statistical Analysis

Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. The tests showed that data are normally distributed (parametric). The Student's test was used to compare the SYM and ASYM groups. The Pearson correlation coefficient was used to determine a significant correlation between the number of cells and the MMP-2 level.[11] The significance level was set at $p \leq .05$. Statistical analysis was performed.

Results

For the MMP-2 immunopositive area percent, the ASYM group showed a statistically significant higher mean area percent than the SYM group (p=.027, Figure 1). For gram-negative cells, the SYM group showed a statistically significantly higher mean number than the ASYM group (p=.003, Figure 2).

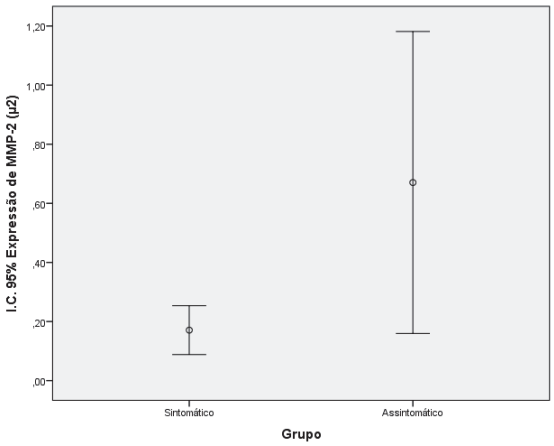

Figura 1 – Quantidade de bactérias Gram-Negativa

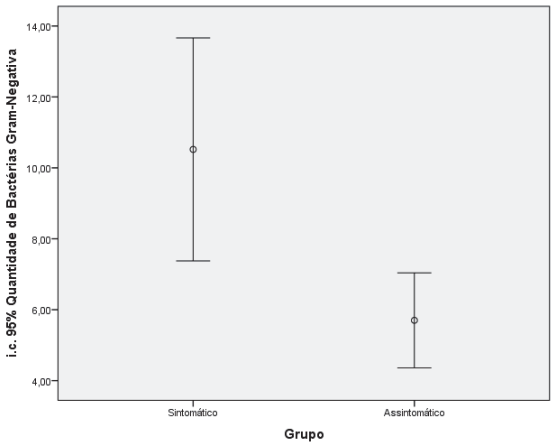

Figura 2 – Expressão de MMP-2

Immunohistochemecally, MMP-2 was expressed mainly by macrophage-like cells, fibroblast-like cells, epithelial cells, and endotelial cells (Figure 3). There was not a statistically significant positive correlation between number of gram-negative cells and the MMP-2 area percent (p=.187).

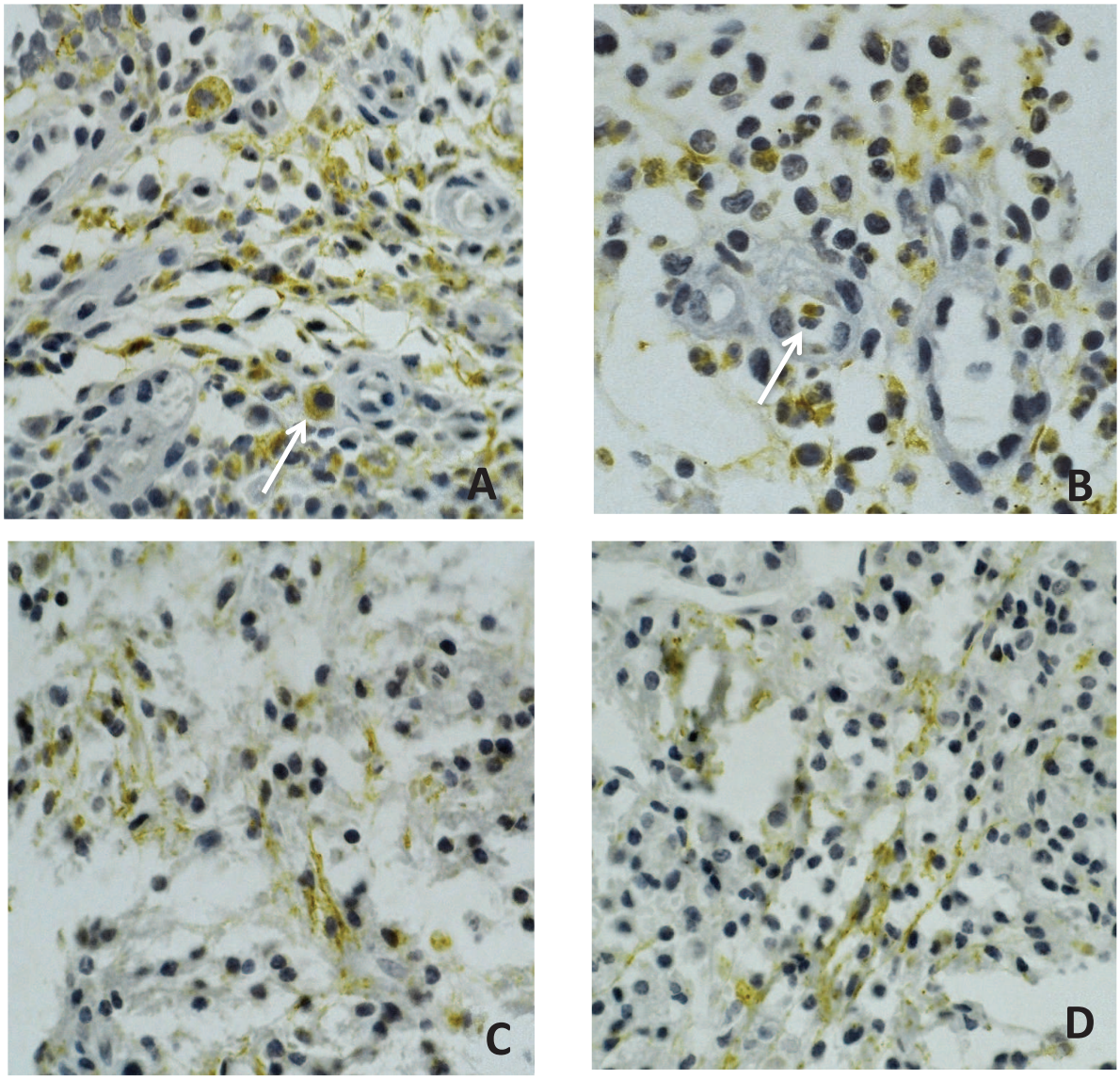

Figura 3 - A: Grupo Assintomático – Área com células inflamatórias imunopositivas para MMP-2, na seta sugestivo de macrófago. B: Grupo Sintomático - Área com células inflamatórias imunopositivas para MMP-2, na seta sugestivo de neutrófilo. C: Grupo Assintomático – Área com células imunopositivas para MMP-2. D: Grupo Assintomático – Área com células imunopositivas para MMP

Discussion

MMPs have been identified in both pulpal and periapical inflammation.[13,6] However, MMP family members are involved in normal physiology and in pathological events that occur in the oral environment.[15]

MMPs play an important role in dental pulp tissue destruction and it has been proposed that MMP-1, MMP-8, MMP-9 and MMP-13 together are involved in jaw cyst expansion.[7,8,16]

Previous studies have shown immunostaining for MMP-2[5,10] In the present study, positive MMP-2 staining was detected in the macrophage-like cells, fibroblast-like cells, , epithelial cells, and endotelial cells.

Increased levels of MMP-2 in human pulp and periodontal ligament all cultures has been associated with porphyromonas endodontalis and gingivalis (black-pigmented bacteroides), which may cause severe periapical abscesses.[17] On the other hand, MMP-2 could be detected in acute apical abscess samples at lower levels than MMP-9.[14] Differents patterns of expression between gelatinases MMP-2 and MMP-9 have been described. Human pulp cells culture have been demonstraded to produce primarily MMP-2 and MMP-9. When IL-1 was introduced, the cells have elevated levels of MMP-2, however IL-1 did not affect MMP-9 level during the 8-day culture period.[17]

The most original findings of this investigation is the pattern of expression of MMP-2 which was the opposite of a recent study using the same methodology, but with MMP-9.[10] MMP-2 and MMP-9 have been described how an importante role during the phase of the periapical lesion development. On the other hand, when the periapical lesion is established, the gelatinases may have a different behavior. In this study, MMP-2 immunostaining in ASYM was higher when compared to the SYM group, however, it may suggest a different role when compared to the MMP-9 in acute periodontitis.

It has been suggested that some gram-negative bacteria are involved with symptomatic lesions.[18] However the presente study did not show a correlation between the gram-negative bacteria and an increase in MMP-2 activity in symptomatic periapical lesions. It can be suggested the balance between gelatinases, probably there are different patterns, when the MMP-9 increases the MMP-2 receives signals to decrease.

For gram-negative bacterias, the SYM group showed a statistically significantly higher mean number than the ASYM group. Based on the results, it can be concluded that gram-negative bacteria may not play an important role in MMP-2 activity when the periapical lesion is symptomatic.

References

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ANEXOS

Parecer do Comitê de Ética

ASSOCIAÇÃO PARANAENSE DE CULTURA - PUCPR

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA Título da Pesquisa: EXPRESSÃO DE MA TRIZ MET ALOPROTEASE EM LESÕES PERIAPICAIS SINTOMÁTICAS E ASSINTOMÁTICAS **Pesquisador:** Everdan Carneiro

Área Temática: Versão: 1 **CAAE:** 24267613.5.0000.0020 **Instituição Proponente:** Pontifícia Universidade Católica do Parana - PUCPR
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER Número do Parecer: 459.509

Data da Relatoria: 13/11/2013

Apresentação do Projeto:

A infecção bacteriana na polpa dentária induz uma resposta inflamatória, que, não sendo tratada, determina a sua destruição e consequ¿ente contaminação do sistema de canais radiculares e túbulos dentinários, desencadeando, por sua vez, infecção no periodonto apical. A persistência desse evento provoca a migração, ativação e a interação coordenada de células imuno-competentes, caracterizando a periodontite apical. A degradação de proteínas da matriz extracelular pelas metaloproteases da matriz (MMPs) ocorre nos processos fisiológicos (turnover) bem como

nos processos patológicos (inflamação, neoplasias, etc).

Objetivo da Pesquisa:

Objetivo Primário: Avaliar a expressão de MMP-2 em lesões periapicais, granulomas e cistos periapicais, verificando as células envolvidas para MMP-2 por meio de marcações de imuno-histoquímica.

Objetivo Secundário: Verificar os níveis de expressão de MMP-2 em lesões

periapicais sintomáticas e assintomáticas.

Avaliação dos Riscos e Benefícios:
Riscos: Não existem riscos envolvidos. O material encontra-se emblocado no Serviço de Patologia e faz parte da rotina desse Serviço a análise microscópica desse tecido coletado na clínica para fins diagnósticos.

Benefícios: Os benefícios para o participante da pesquisa é que existirá além do exame anatomopatológico de rotina, um exame imunohistoquímico, sem riscos e necessidade de outras coletas.

Comentários e Considerações sobre a Pesquisa:

Um total de 20 amostras de tecidos retirados de cirurgias paraendodônticas, realizadas pelo Serviço de Endodontia da PUCPR, serão coletadas encaminhadas ao Serviço de Patologia para análise e laudo de anatomopatológico. Os resultados serão comparados conforme protocolo estabelecido no projeto de pesquisa e analisados estatisticamente.

Considerações sobre os Termos de apresentação obrigatória:

O TCLE está presente e escrito de maneira adequada. Sugere-se que o pesquisador acrescente a seguinte frase no termo: "Em caso de reclamação ou qualquer tipo de denuncia sobre este estudo devo ligar para o CEP PUCPR (41) 3271-2292 ou mandar um email para nep@pucpr.br."

Recomendações:

Seguir recomendação de adequação do TCLE.

Conclusões ou Pendências e Lista de Inadequações:

O presente projeto de pesquisa está aprovado no quesito ético.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Lembramos aos senhores pesquisadores que, no cumprimento da Resolução 466/2012, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios anuais sobre o andamento do estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de

eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do estudo.

Eventuais modificações ou ementas ao protocolo devem ser apresentadas ao CEP-PUCPR de forma clara e sucinta, identificando a parte do protocolo a ser modificado e as suas justificativas.

Se a pesquisa, ou parte dela for realizada em outras instituições, cabe ao pesquisador não iniciá-la antes de receber a autorização formal para a sua realização. O documento que autoriza o início da pesquisa deve ser carimbado e assinado pelo responsável da instituição e deve ser mantido em poder do pesquisador responsável, podendo ser requerido por este CEP em qualquer tempo.

CURITIBA, 18 de Novembro de 2013

Assinador por:

NAIM AKEL FILHO (Coordenador)

_____

Endereço: Rua Imaculada Conceição 1155 **Bairro:** Prado Velho **CEP:** 80.215-901 **UF:** PR **Município:** CURITIBA **Telefone:** (41)3271-2292 **Fax:** (41)3271-2292 **E-mail:** nep@pucpr.br

Análise Estatística

Resultados

Testes de Normalidade

Grupo		Kolmogorov-Smirnov[a] Estatística	df	Valor p	Shapiro-Wilk Estatística	df	Valor p
Expressão de MMP-2 (µ2)	Sintomático	,159	10	,200*	,965	10	,844
	Assintomático	,256	10	,062	,850	10	,059
Quantidade de Bactérias Gram-Negativa	Sintomático	,152	10	,200*	,914	10	,306
	Assintomático	,180	10	,200*	,904	10	,242

*. Este é um limite inferior da significância verdadeira.

a. Correlação de Significância de Lilliefors

Descritivos

		N	Média	Desvio Padrão	Erro Padrão	média Limite inferior	média Limite superior	Mínimo	Máximo
Expressão de MMP-2 (µ2)	Sintomático	10	0,17	0,12	0,04	0,09	0,25	0,01	0,37
	Assintomático	10	0,67	0,71	0,23	0,16	1,18	0,02	2,21
	Total	20	0,42	0,56	0,13	0,16	0,68	0,01	2,21
Quantidade de Bactérias Gram-Negativa	Sintomático	10	10,52	4,40	1,39	7,38	13,66	6,00	19,20
	Assintomático	10	5,70	1,87	0,59	4,36	7,04	3,00	8,00
	Total	20	8,11	4,11	0,92	6,18	10,04	3,00	19,20

Teste de Homogeneidade de Variâncias

	Estatística de Levene	df1	df2	Valor p
Expressão de MMP-2 (µ2)	13,884	1	18	0,0015
Quantidade de Bactérias Gram-Negativa	6,034	1	18	0,0244

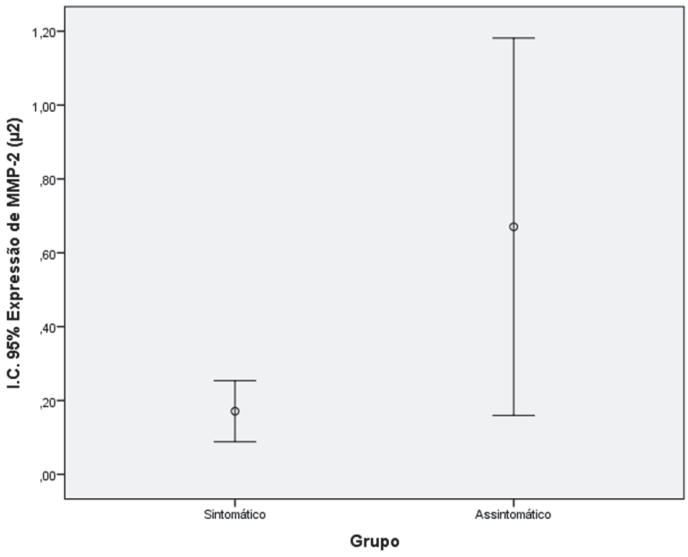
Teste de amostras independentes

		teste-t para Igualdade de Médias t	df	Valor p (bilateral)	Valor p (unilateral)	Diferença média	Erro padrão de diferença	95% Intervalo de Confiança da Diferença Inferior	Superior	Potência observada[1]
Expressão de MMP-2 (µ2)	Variâncias iguais não assumidas	-2,1832	9,4715	0,0554	0,0277	-0,4995	0,2288	-1,0132	0,0142	0,5423
Quantidade de Bactérias Gram-Negativa	Variâncias iguais não assumidas	3,1895	12,1657	0,0077	0,0038	4,8200	1,5112	1,5323	8,1077	0,8544

(1). Calculado usando alfa = ,05

Valor p < 0,05 (teste unilateral) indica diferença estatisticamente significante entre os dois grupos

1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1,00
,80
,60
,40
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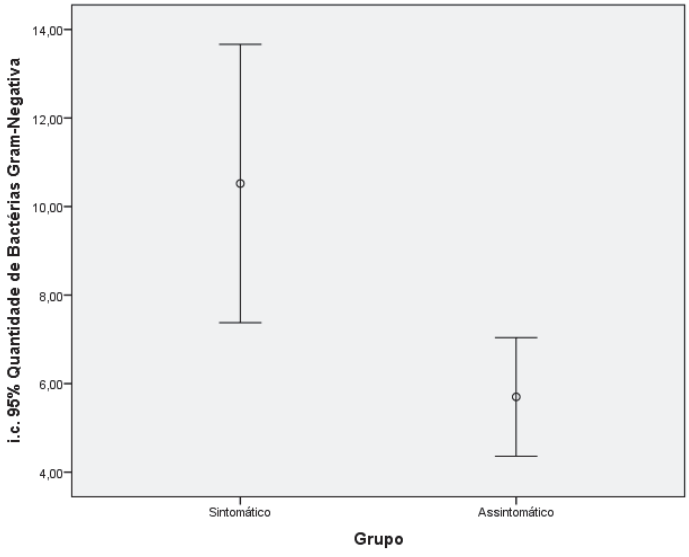
,00
I.C. 95% Expressão de MMP-2 (µ2)
Sintomático
Assintomático
Grupo

2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12,00
10,00
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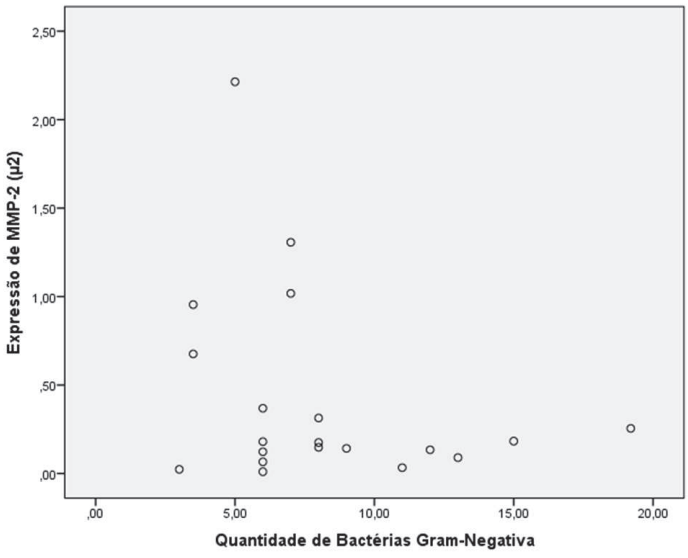
6,00
4,00
i.c. 95% Quantidade de Bactérias Gram-Negativa
Sintomático
Assintomático
Grupo

3


2,50
2,00
1,50
1,00
,50
,00
Expressão de MMP-2 (µ2)
,00
5,00
10,00
15,00
20,00
Quantidade de Bactérias Gram-Negativa

CORRELAÇÃO GERAL

Correlações

		Expressão de MMP-2 (µ2)	Quantidade de Bactérias Gram-Negativa
Expressão de MMP-2 (µ2)	Correlação de Pearson	1	-,308
	Valor p		,187
	N	20	20
Quantidade de Bactérias Gram-Negativa	Correlação de Pearson	-,308	1
	Valor p	,187	
	N	20	20

CORRELAÇÃO DENTRO DO GRUPO SINTOMÁTICO

Correlações

		Expressão de MMP-2 (µ2)	Quantidade de Bactérias Gram-Negativa
Expressão de MMP-2 (µ2)	Correlação de Pearson	1	-,014
	Valor p		,969
	N	10	10
Quantidade de Bactérias Gram-Negativa	Correlação de Pearson	-,014	1
	Valor p	,969	
	N	10	10

CORRELAÇÃO DENTRO DO GRUPO 2

Correlações

		Expressão de MMP-2 (µ2)	Quantidade de Bactérias Gram-Negativa
Expressão de MMP-2 (µ2)	Correlação de Pearson	1	-,115
	Valor p		,753
	N	10	10
Quantidade de Bactérias Gram-Negativa	Correlação de Pearson	-,115	1
	Valor p	,753	
	N	10	10

1

1 GRAM

Número da lâmina	Área de GRAM	Grupo
2011012-1	3	2
2011012-2	8	2
2011012-3	9	2
2011012-4	7	2
2011012-5	3	2
2011012-6	7	2
2011012-7	3	2
2011012-8	9	2
2011012-9	6	2
2011012-10	5	2
2011012-média	6	2

2

Número da lâmina	Área de GRAM	Grupo
2013038-1	7	2
2013038-2	4	2
2013038-3	6	2
2013038-4	10	2
2013038-5	9	2
2013038-6	9	2
2013038-7	6	2
2013038-8	5	2
2013038-9	3	2
2013038-10	7	2
2013038-média	6,6	2

3

Número da lâmina	Área de GRAM	Grupo
2012025-1	8	2
2012025-2	6	2
2012025-3	5	2
2012025-4	8	2
2012025-5	7	2
2012025-6	10	2
2012025-7	8	2
2012025-8	11	2
2012025-9	7	2
2012025-10	11	2
2012025-média	8,1	2

4

Número da lâmina	Área de GRAM	Grupo
2013013-1	5	2
2013013-2	2	2
2013013-3	1	2
2013013-4	2	2
2013013-5	4	2
2013013-6	2	2
2013013-7	2	2
2013013-8	3	2
2013013-9	1	2
2013013-10	3	2
2013013-média	2,5	2

1

Número da lâmina	Área de GRAM	Grupo
2013047-1	9	2
2013047-2	8	2
2013047-3	13	2
2013047-4	4	2
2013047-5	11	2
2013047-6	7	2
2013047-7	8	2
2013047-8	4	2
2013047-9	7	2
2013047-10	5	2
2013047-média	7,6	2

2

Número da lâmina	Área de GRAM	Grupo
2013050-1	2	2
2013050-2	6	2
2013050-3	9	2
2013050-4	2	2
2013050-5	4	2
2013050-6	8	2
2013050-7	6	2
2013050-8	2	2
2013050-9	4	2
2013050-10	5	2
2013050-média	4,8	2

3

4

5

Número da lâmina	Área de GRAM	Grupo
2013069-1	3	2
2013069-2	5	2
2013069-3	3	2
2013069-4	6	2
2013069-5	3	2
2013069-6	2	2
2013069-7	4	2
2013069-8	4	2
2013069-9	2	2
2013069-10	3	2
2013069-média	4	2

1

Número da lâmina	Área de GRAM	Grupo
2013072-1	3	2
2013072-2	3	2
2013072-3	7	2
2013072-4	5	2
2013072-5	3	2
2013072-6	5	2
2013072-7	4	2
2013072-8	2	2
2013072-9	1	2
2013072-10	2	2
2013072-média	4	2

2

Número da lâmina	Área de GRAM	Grupo
2013073-1	10	2
2013073-2	12	2
2013073-3	7	2
2013073-4	6	2
2013073-5	8	2
2013073-6	5	2
2013073-7	7	2
2013073-8	3	2
2013073-9	4	2
2013073-10	11	2
2013073-média	7,3	2

3

4

5

Número da lâmina	Área de GRAM	Grupo
2013048-1	8	2
2013048-2	8	2
2013048-3	10	2
2013048-4	7	2
2013048-5	6	2
2013048-6	2	2
2013048-7	7	2
2013048-8	4	2
2013048-9	4	2
2013048-10	3	2
2013048-média	5,9	2

1

2 MMP

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2011012-1	0,44292092	2
2011012-2	0,10522959	2
2011012-3	0,61766583	2
2011012-4	0,2002551	2
2011012-5	0,33003825	2
2011012-6	0,12308674	2
2011012-7	0,12308675	2
2011012-8	0,12308676	2
2011012-9	0,12308677	2
2011012-10	0,12308678	2
2011012-média	0,231154349	2

3

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013038-1	2,2228954	2
2013038-2	7,561862	2
2013038-3	2,7101402	2
2013038-4	5,5318875	2
2013038-5	6,5443239	2
2013038-6	6,6036353	2
2013038-7	3,2410715	2
2013038-8	5,3017978	2
2013038-9	2,9639668	2
2013038-10	3,6801658	2
2013038-média	4,63617462	2

4

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2012025-1	0,20567602	2
2012025-2	0,14604591	2
2012025-3	0,04432397	2
2012025-4	0,1916454	2
2012025-5	0,19515306	2
2012025-6	0,14987245	2
2012025-7	0,07397959	2
2012025-8	0,24585459	2
2012025-9	0,07684949	2
2012025-10	0,0851403	2
2012025-média	0,141454078	2

1

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013013-1	0,09534438	2
2013013-2	0,00892857	2
2013013-3	0,02710459	2
2013013-4	0,00510204	2
2013013-5	0,02008928	2
2013013-6	0,03794642	2
2013013-7	0,03858418	2
2013013-8	0,01116071	2
2013013-9	0,00892857	2
2013013-10	0,03156887	2
2013013-média	0,028475761	2

2

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013047-1	0,10299744	2
2013047-2	0,23309949	2
2013047-3	0,27232143	2
2013047-4	0,04368622	2
2013047-5	0,10044643	2
2013047-6	0,38169643	2
2013047-7	0,47672191	2
2013047-8	0,21301021	2
2013047-9	0,13679847	2
2013047-10	0,10267857	2
2013047-média	0,20634566	2

3

4

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013050-1	18,083227	2
2013050-2	0,5194515	2
2013050-3	1,9620535	2
2013050-4	23,899553	2
2013050-5	21,390944	2
2013050-6	0,92793363	2
2013050-7	2,7209821	2
2013050-8	2,4661989	2
2013050-9	0,96715558	2
2013050-10	1,6167091	2
2013050-média	7,455420831	2

1

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013069-1	1,5373087	2
2013069-2	0,39540815	2
2013069-3	1,4681122	2
2013069-4	1,3711735	2
2013069-5	1,5232780	2
2013069-6	0,7225765	2
2013069-7	1,1868622	2
2013069-8	0,45503825	2
2013069-9	0,35140306	2
2013069-10	0,21492347	2
2013069-média	0,922608403	2

2

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013072-1	0,15146683	2
2013072-2	0,88903058	2
2013072-3	1,7155612	2
2013072-4	0,52168369	2
2013072-5	0,34056121	2
2013072-6	0,19770408	2
2013072-7	0,57621175	2
2013072-8	0,77582908	2
2013072-9	2,3281250	2
2013072-10	0,88488519	2
2013072-média	0,838105861	2

3

4

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013073-1	0,44897959	2
2013073-2	1,2975128	2
2013073-3	1,3013393	2
2013073-4	2,327806	2
2013073-5	0,79464287	2
2013073-6	1,5121173	2
2013073-7	1,5440050	2
2013073-8	1,3121811	2
2013073-9	1,7975128	2
2013073-10	0,36096939	2
2013073-média	1,269706615	2

Número da lâmina	Área da MMP-2 (micrômetros quadrados)	Grupo
2013048-1	0,05293367	2
2013048-2	0,0041454	2
2013048-3	0,01307397	2
2013048-4	0,01690051	2
2013048-5	0,11575255	2
2013048-6	0,07908163	2
2013048-7	0,08067601	2
2013048-8	0,14285713	2
2013048-9	0,01849489	2
2013048-10	0,12978315	2
2013048-média	0,065369891	2

Legenda:

Grupo 1- sintomático

Grupo 2- assintomático

1 Resultados Finais

ExpressãodeMMP2µ2	QuantidadedeBactériasGramNegativa	Grupo
0,26	19,20	1
0,09	13,00	1
0,18	15,00	1
0,37	6,00	1
0,01	6,00	1
0,18	6,00	1
0,31	8,00	1
0,13	12,00	1
0,03	11,00	1
0,14	9,00	1
0,12	6,00	2
1,02	7,00	2
0,15	8,00	2
0,02	3,00	2
0,17	8,00	2
2,21	5,00	2
0,95	3,50	2
0,68	3,50	2
1,31	7,00	2
0,07	6,00	2

2

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a. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section “c,” below), or sentences with little to no transition within a paragraph.

b. Keep to the point. The subject of the sentence should support the subject of the paragraph For example, the introduction of authors’ names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, “In 1983, Langeland et al, reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals” can be edited to: “Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al, 1983).” In this example, the paragraph’s subject is sodium hypochlorite and sentences should focus on this subject.

c. Sentences are stronger when written in the active voice, that is, the subject performs the action. Passive sentences are identified by the use of passive verbs such as “was,” “were,” “could,” etc. For example: “Dexamethasone was found in this study to be a factor that was associated with reduced inflammation,” can be edited to: “Our results demonstrated that dexamethasone reduced inflammation.” Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

d. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus, or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)

e. Use parallel construction to express related ideas. For example, the sentence, “Formerly, endodontics was taught by hand instrumentation, while now rotary

instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.

f. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."

g. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.

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