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ASSOCIAÇÃO ENTRE PERIODONTITE APICAL E NÍVEIS SÉRICOS DE PROTEÍNA C-REATIVA EM UMA POPULAÇÃO RURAL DO SUL DO BRASIL

Santa Maria, RS, Brasil
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, Ênfase em Endodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial à obtenção do título de **Mestre em Ciências Odontológicas.**

Orientador: Prof. Dr. Carlos Alexandre Souza Bier

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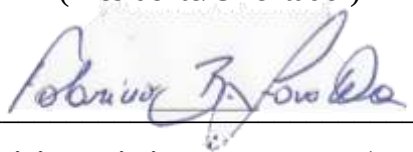

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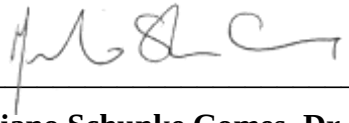

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2021

DEDICATÓRIA

*Aos meus pais, **Roselei** Keller Salvador e **João** Raimundo Cruz da Cruz, por serem meu alicerce e por compartilharem dos sonhos junto comigo e estarem ao meu lado na realização deles.*

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RESUMO

ASSOCIAÇÃO ENTRE PERIODONTITE APICAL E NÍVEIS SÉRICOS DE PROTEÍNA C-REATIVA EM UMA POPULAÇÃO RURAL DO SUL DO BRASIL

AUTORA: Anna Luiza Salvador Da Cruz
ORIENTADOR: Carlos Alexandre Souza Bier

A periodontite apical (PA) é um processo inflamatório na região apical de um elemento dentário resultante da colonização de microrganismos no sistema de canais radiculares. A proteína C-reativa (PCR) é um reagente de fase aguda cujos níveis se elevam em decorrência de uma inflamação aguda ou crônica, além de ser considerado um mediador inflamatório sistêmico e um biomarcador na previsão de doenças cardiovasculares. O objetivo deste estudo transversal foi avaliar a associação entre a periodontite apical e a elevação dos níveis séricos de proteína C-reativa em uma amostra da população da zona rural do município de Rosário do Sul, RS. A amostra de 515 indivíduos foi obtida a partir de um levantamento epidemiológico realizado em Rosário do Sul, no período entre março de 2015 a maio de 2016. Os indivíduos que apresentavam periodontite apical foram divididos em grupos em relação a severidade e extensão das lesões, sendo classificados como baixo nível os indivíduos com menos de 10% dos dentes presentes com PAI 2 ou 3 e 4 ou 5 em menos de 10% dos detes presentes e alto nível os indivíduos com mais de 10% dos dentes presentes com PAI 4 ou 5. Foram consideradas na análise variáveis referentes ao sexo, idade, fumo, variável socioeconômica, IMC, pressão arterial, hemoglobina glicada, uso de medicações e características periodontais. A análise estatística foi realizada utilizando programa Stata 14.0. Foi realizada análise descritiva dos dados, regressão linear e regressão logitica binária. A comparação dos grupos foi vista através do do teste Qui quadrado, Mann Whitney, Kruskal-Wallis, post hoc de Dunn e teste t de Student. Os resultados da análise ajustada demonstraram que indivíduos com grau mais avançado de periodontite apical apresentaram níveis elevados de PCR quando comparados àqueles com níveis baixos de PA ($p < 0,05$). Além disso, os indivíduos com PA tiveram uma chance de 2,3 vezes maior de ter PCR acima de 5 mg / L quando comparado aos indivíduos sem PA (Odds Ratio = 2,30; IC de 95%: 1,31 a 4,05). Os resultados demonstraram uma associação entre elevados níveis séricos de PCR e periodontite apical, sexo e IMC. Portanto, de acordo com o presente estudo, a periodontite apical foi associada a níveis mais elevados de PCR em indivíduos residentes em área rural e pode influenciar biomarcadores sistêmicos e afetar a saúde geral do paciente.

Palavras-chave: Periodontite apical. Proteína C-Reativa. População rural. Saúde oral. Inflamação. Doença cardiovascular.

ABSTRACT

ASSOCIATION BETWEEN APICAL PERIODONTITIS AND SERUM C-REACTIVE PROTEIN LEVELS IN A RURAL POPULATION IN SOUTHERN BRAZIL

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ADVISOR: Carlos Alexandre Souza Bier

Apical periodontitis (AP) is an inflammatory process in the apical region of a tooth element resulting from the colonization of microorganisms in the root canal system. C-reactive protein (CRP) is an acute-phase reagent whose levels increase as a result of acute or chronic inflammation, in addition to being considered a systemic inflammatory mediator and a biomarker in predicting cardiovascular disease. The aim of this cross-sectional study was to evaluate the association between apical periodontitis and increased serum levels of C-reactive protein in a sample of the rural population of the municipality of Rosário do Sul, RS. The sample of 515 individuals was obtained from an epidemiological survey carried out in Rosário do Sul, in the period between March 2015 and May 2016. Individuals with apical periodontitis were divided into groups in relation to the severity and extent of the lesions. classified as low level individuals with less than 10% of teeth present with PAI 2 or 3 and 4 or 5 in less than 10% of teeth present and high level individuals with more than 10% of teeth present with PAI 4 or 5. Variables related to sex, age, smoking, socioeconomic variable, BMI, blood pressure, glycated hemoglobin, use of medication and periodontal characteristics were considered in the analysis. Statistical analysis was performed using the Stata 14.0 program. Descriptive data analysis, linear regression and binary logistic regression were performed. The comparison of groups was seen using the Chi-square test, Mann Whitney, Kruskal-Wallis, Dunn's post hoc and Student's t test. The results of the adjusted analysis showed that individuals with a more advanced degree of apical periodontitis had higher levels of CRP when compared to those with low levels of BP ($p < 0.05$). In addition, individuals with BP had a 2.4 times greater chance of having CRP above 5 mg/L when compared to individuals without BP (Odds Ratio = 2.30; 95% CI: 1.31 to 4, 05). The results demonstrated an association between high serum CRP levels and apical periodontitis, gender and BMI. Therefore, according to the present study, apical periodontitis was associated with higher levels of CRP in individuals living in rural areas and may influence systemic biomarkers and affect the patient's general health.

Keywords: Apical Periodontitis. C-Reactive Protein. Rural population. Mouth Diseases. Inflammation. Cardiovascular Diseases.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

CEP	Comitê de Ética em Pesquisa
IBGE	Instituto Brasileiro de Geografia e Estatística
LP	Lesão Periapical
PAI	The periapical index
PCR	Proteína C-Reativa
TCLE	Termo de Consentimento Livre e Esclarecido
UFSM	Universidade Federal de Santa Maria
PA	Periodontite apical

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SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

A Periodontite apical (PA) é um processo inflamatório que circunda o ápice de uma raiz dentária e pode se manifestar de forma aguda ou crônica (GUTMANN et al., 2009; RICCUCI, SIQUEIRA, 2013). A etiologia desta condição é a colonização de microrganismos no sistema de canais radiculares, seguido de necrose pulpar, até que a migração dos microrganismos para a região apical e estabelecimento da condição infecciosa. (COLIC et al., 2009; SIQUEIRA, 2011).

Para que o processo de necrose pulpar aconteça é necessária uma agressão persistente presente, assim a resposta inflamatória também será persistente (LOPES, SIQUEIRA, 2015). A cárie dentária é geralmente o principal agressor, pois permite a entrada de microrganismos através da câmara pulpar e consequentemente, para o canal radicular (RICUCCI, 2013). A lesão de cárie quando não tratada progride até atingir o tecido pulpar, causar alterações inflamatórias locais e inflamação crônica da polpa, o que pode levar o tecido pulpar a necrose e migração das bactérias para a região periapical e causar um envolvimento sistêmico tanto pela bacteremia quanto pelo estímulo do processo inflamatório na corrente sanguínea que permitirá a liberação de citocinas (ABBOTT, 2007). Quando a infecção atinge o periápice há um estímulo dos mediadores inflamatórios, estes então causam mudanças na estrutura óssea periapical, resultando em reabsorção óssea observada através de radiolúcidez em radiografias (DE SA et al., 2003; NAIR, 2004)

A manifestação da PA pode acontecer de várias formas, desde assintomáticas com apenas sinais radiográficos a grandes sinais clínicos de inflamação. Essa manifestação dependerá da intensidade da agressão (LOPES, SIQUEIRA, 2015). Em casos em que há manifestações clinicas características de um processo inflamatório agudo, como dor, edema, a intervenção será necessária e geralmente, com urgência. Entretanto, em casos assintomáticos, mesmo que também necessitem intervenção obrigatória, estas lesões podem passar despercebidas e continuar afetando a saúde geral do paciente (GEORGIOU et al, 2019).

O diagnóstico de PA é realizado através de exames de imagem e exame clínico (Abott, 2004). Dentre os exames de imagem mais utilizados estão a radiografia periapical, a panorâmica e a tomografia computadorizada de feixe cônico (TCFC). Em relação a acurácia para avaliação da PA, a TCFC é a melhor opção, seguida da radiografia periapical e por último a panorâmica (ESTRELA et al., 2008). A radiografia panorâmica pode não ser eficaz para diagnóstico de da

região periapical para fins de triagem (RidaoSacie et al. 2007), bem como a TCFC pode ser associada a diagnóstico equivocado quando comparado a radiografia periapical (KRUSE et al, 2019). Portanto, em estudos epidemiológicos, a CBFC não deve ser o método de escolha para análise radiográfica, de acordo com as evidências e os princípios de ALARA.

O índice PAI "The periapical índex" é utilizado na avaliação do status periapical dos dentes em radiografias periapicais e panorâmicas (ORSTAVIK et al, 1986). Este sistema fornece uma escala ordinal de 5 pontos, variando de 1 (saudável) a 5 (periodontite severa com características exacerbadas). A sua validade é baseada no uso de radiografias de dentes com diagnósticos histológicos verificados (BRYNOLF, 1967)

Há uma crescente preocupação no sentido de que uma infecção, mesmo que localizada, como a periodontite apical possa afetar a saúde geral do paciente, pois os microorganismos envolvidos nas lesões endodônticas quando atingem a corrente sanguínea podem liberar antígenos sobre o tecido periapical, iniciando e sustentando uma resposta inflamatória que leva o osso a reabsorção e destruição do ligamento periodontal (DE SA et al. 2003, NAIR 2004). O sistema imune inato quando detecta alguma agressão tecidual ou infecção, estimula componentes celulares e moleculares que levam a uma reação do organismo chamada de inflamação. Esse processo inflamatório induz uma resposta inflamatória de fase aguda inespecífica dirigido por células e mediadores de inflamação. Citocinas pró-inflamatórias, como interleucina-1b (IL-1b),IL-6, IL-11, IL-17 e fator de necrose tumoral alfa (TNF-a), são secretados por diferentes células envolvidas na a resposta imune periapical. Essas células e mediadores inflamatórios desempenham um papel importante na patogênese da periodontite apical crônica (AJUZ et al. 2014). Conforme o processo de reabsorção óssea avança e o processo inflamatório estimula a liberação de citocinas na corrente sanguínea, a área se torna um reservatório para marcadores inflamatórios como citocinas, anticorpos e proteína C-reativa (PCR) (GOMES et al. 2013).

As proteínas de fase aguda (PFA) são produzidas pelos hepatócitos no fígado e são importantes parâmetros na resposta sistêmica diante da presença e evolução inflamatória e/ou infecciosa pois em processo de doença ocorre a alteração em suas concentrações, seja um decréscimo (PFA negativas) ou o aumento (PFA positiva) (CRISPE, 2016). A proteína C-reativa faz parte do grupo das PFA positiva (GABAY; KUSHNER, 1999; GERMOLEC et al., 2018). Essa alteração na concentração das PFA é estimulada por citocinas pró-inflamatórias como interleucina-1 (IL-1), IL-2, IL-6 e fator de necrose tumoral alfa (TNF-α) (GABAY, C;

KUSHNER, 1999) e essas possuem um papel essencial nas vias de inflamação local e sistêmica (BAKER, 2000; KANTARCI; HASTURK; VAN DYKE, 2015; KIM; AMAR, 2006)

Por isso, as lesões periapiacais e a doença periodontal podem afetar a saúde geral do paciente e até mesmo serem fatores predisponentes de outras condições, como por exemplo a doença cardiovascular (GOMES et al., 2013). Portanto, o tratamento endodôntico e o tratamento das demais condições tem uma importância na promoção de saúde do paciente. Em especial, a endodontia é a principal forma conservadora de tratar a infecção do canal e a lesão inflamatória, mantendo o dente em função e com saúde (LOPES, SIQUEIRA, 2015)

A proteína C-reativa (PCR) é um reagente de fase aguda que participa da resposta sistêmica à inflamação e é produzido principalmente por hepatócitos. Sua concentração plasmática aumenta durante estados inflamatórios agudos e crônicos (AGUIAR, 2013). A elevação dos níveis de PCR possui um papel fisiológico importante como mediador inflamatório (MCFADYEN, 2018) e indica principalmente a presença de inflamação, entretanto podem indicar também a presença de processo reumático, presença de tumores malignos e reação medicamentosa (LANDRY, 2017) e tem papel na monitoração da progressão de doenças crônicas (PEPYS, HIRSCHFIELD, 2003). Além disso, elevados níveis de PCR são relacionados ao aumento no risco de eventos cardiovasculares agudos e é considerado um biomarcador padrão na previsão de doença cardiovascular, pois pode auxiliar na formação de placas escleróticas e oclusão vascular (AVAN, et al, 2018; TSIMIKAS et al., 2006; CASAS et al., 2008).

A PCR exerce um importante papel no desenvolvimento e progressão da aterosclerose através do aumento da expressão de moléculas de adesão, proteína quimiotática de monócitos do tipo 1 (MCP-1), endotelina e inibidor do ativador de plasminogênio. A PCR também reduz a biodisponibilidade de óxido nítrico, além de induzir a diferenciação dos monócitos em macrófagos (VERMA et al., 2002; VENUGOPAL et al, 2002; DADU et al, 2012).

De acordo com a American Heart Association (AHA) sobre marcadores inflamatórios em doenças cardiovasculares ficou estabelecido que níveis menores de 1 mg / L representam baixo risco, 1-3 mg / L risco médio e alto risco acima de 3 mg/ L. (PEARSON et al., 2003).

A ateroescleorose é definida como um processo inflamatório crônico e progressivo que atinge principalmente a camada interna de artérias de médio e grande calibre (ROOS,1999). São fatores de risco para o desenvolvimento das placas a dislipidemia, a hipertensão arterial e o tabagismo, pois estes são essenciais no processo inicial da doença, ou seja, na lesão endotelial

que é caracterizada pela diminuição da produção de óxido nítrico (NO) e liberação de vários mediadores inflamatórios e moléculas quimioatraentes em resposta à injúria tecidual, desencadeada pelo acúmulo de LDL na parede das artérias. Além disso, o endotélio arterial a partir da injuria, exercerá propriedades pró-coagulantes ao invés de anticoagulantes (HANSSON, 2005; ROOS, 1999). As principais complicações da aterosclerose são o infarto agudo do miocárdio e o acidente Vascular Cerebral (AVC) (MACKMAN, 2008).

As doenças cardiovasculares e circulatórias são responsáveis por grande parte das mortes mundias. Em 2010, a isquemia e acidente vascular cerebral levaram a 12,9 milhões de mortes, o que significa quase 25% de todas as mortes em todo o mundo (LOZANO et al., 2012). A etiologia da doença cardiovascular é a interação da predisposição genética e fatores ambientais externos, tais como tabagismo sexo, diabetes, hipertensão, marcadores sorológicos de inflamação e obesidade. Entretanto, além destes fatores, as doenças orais também estão sendo consideradas como fatores de risco adicionais as demais, devido as características de manifestação da inflamação na repercussão sistêmica e produção de mediadores inflamatórios presentes nas doenças orais que podem influenciar nas doenças cardiovasculares. (JANKET et al., 2015)

Entender que as condições orais não se restringem somente a cavidade bucal, levou a um grande número de pesquisas desde o século XIX, onde começou-se a pensar sobre as possíveis consequências sistêmicas de dentes infectados (O'REILLY, CLAFFEY, 2000). Em 1967 Boucher et al. avaliou a repercussão sistêmica nos níveis de PCR de diversas infeções orais e mostrou que haviam diferenças entre os pacientes com alguma infecção comparado aos que não apresentavam alteração. Além disso, quando o dente era extraído e os níveis de mediadores avaliados novamente, percebia-se uma redução nos níveis.

Na área da periodontia a relação entre a infecção local e a saúde sistêmica do paciente tem sido bem estabelecida, através da associação entre periodontite e doenças cardiovasculares ateroescleróticas (BEUKERS et al, 2017), periodontite e doença de Parkinson (CHEN et al, 2017), diabetes, intercorrências na gravidez e doenças pulmonares (SABHARWAl, 2000). Para explicar a associação entre estas doenças existem dois mecanismos. O primeiro, no qual as bactérias da placa subgengival podem se deslocar para a circulação sistêmica e promover uma resposta inflamatória (KEBSCHULL, 2010). O segundo explicado pelas citocinas pró-inflamatórias produzidas localmente em lesões periodontais possam entrar na corrente sanguínea, resultando em inflamação sistêmicas caracterizada por níveis elevados de mediadores inflamatórios, como a proteína C reativa (CRP) (PARASKEVAS, 2008). A

inflamação sistêmica, por sua vez, acelera o progresso da aterosclerose e aumenta o risco de ruptura das placas. (LIBBY, 2012).

Segundo Segura Egea et al (2015), os processos inflamatórios periodontais e endodônticos têm semelhanças que devem ser consideradas. Ambos são infecções crônicas da cavidade oral, ambos são infecções polimicrobianas com predominância de bactéticas gram negativas anaeróbias (SIQUEIRA, ROÇAS 2014) e ambos possuem níveis elevados de citocinas locais, tanto do fluido crevicular, quanto dos tecidos periapicais que podem ser liberados sistemicamente (CAPLAN 2004, CAPLAN et al., 2006). Portanto, visto que a doença periodontal possui influência sistêmica e possui similaridade com a doença periapical, é possível que essa também cause alterações a nível sistêmico.

As infecções crônicas como a periodontite apical e as infecções periodontais, apesar da diferença na etiologia, contêm semelhanças na presença de patógenos de bactérias anaeróbias gram-negativas e elevação do nível de citocinas. Algumas evidências foram estabelecidas em relação à produção dessas citocinas pela polpa inflamada e tecido granulomatoso periapical (CAPLAN, 2004; BARKHORDAR, 1999; BASCONES et al, 2000). Muitas publicações relatam a associação entre a doença periodontal e doenças sistêmicas, como a doença cardiovascular (CARRION, 2012). Recentemente, um estudo nesse contexto, relatou a associação entre PCR e fibrinogênio com a doença periodontal sendo que estes mediadores aumentaram com a gravidade da periodontite, demonstrando uma associação diretamente proporcional entre as variáveis. (ANDREU, 2021). Além disso, há redução nos níveis dos mediadores quando a terapia periodontal é realizada (VIDAL et al. 2016).

Considerando que área periapical acometida pela reabsorção óssea decorrente da infecção endodôntica contém marcadores inflamatórios como citocinas, anticorpos, metabólitos, proteína C-reativa, fator de necrose tumoral alfa (TNF), interleucinas, interglobulinas (STASHENKO et al, 1998; GOMES et al, 2013), sabe-se que essas citocinas podem ser liberadas na circulação sistêmica devido à proximidade anatômica de infecções endodônticas e periodontais com a corrente sanguínea (THOMAS et al, 2012, DOYLE et al. 2007) o que pode induzir ou perpetuar um status inflamatório sistêmico crônico elevado (CAPLAN et al. 2006).

Em 2006, Caplan et al. relataram que homens com menos de 40 anos com periodontite apical sofriam de doença coronariana de início mais precoce do que homens sem PA. Além disso em um estudo no qual 508 pacientes foram submetidos a angiografia coronariana, a

presença de lesões endodônticas foi significativamente associada a doença cardíaca coronária (LILJESTRAND, 2016). Frisk (2006) não observou relação significativa entre PA e doença cardíaca coronária, entretanto este resultado pode ter sido influenciado pela amostra representada por mulheres, cja modulação cardiovascular pode ser alterada hormonalmente.

Kimak et al (2015) realizaram um estudo para avaliação de lipídios, lipoproteínas e marcadores inflamatórios em pacientes com periodontite crônica. Houve relações significativas entre a presença de lesão periapical e os níveis de marcadores inflamatórios como a PCR em pacientes com menos de 50 anos. Em pacientes com mais de 50 anos, além da PCR houve diferença estatisticamente significativa também para os mediadores IL-6, TNF, além disso, os pacientes que apresentavam lesões periapicais mais extensas apresentaram os parâmetros dos mediadores inflamatórios mais elevados.

Em 2013 uma revisão sistemática foi realizada com a finalidade de avaliar se a periodontite apical seria capaz de modificar os níveis de mediadores inflamatórios sistêmicos como IgA, IgG, IgM, proteína C reativa (PCR). Os estudos incluidos mostravam uma diferença estatisticamente significativa nos níveis de interleucina (IL) IL-1, IL-2, IL-6, PCR, interleucina (IL) -1 em pacientes com periodontite apical, comparado aos controles, quando analisados separadamente. Os resultados deste estudo demonstraram associação significativa somente para IgA, IgG e IgM em comparação com controles saudáveis. Os níveis séricos de PCR, IgA, IgE, IgG e IgM não foram estatisticamente significativos entre pacientes com PA antes e após a intervenção do tratamento, provavelmente pelo número de estudos incluídos. Esses achados sugeriram que a PA poderia contribuir para uma resposta imune não confinada à lesão localizada, levando ao aumento da inflamação sistêmica (GOMES et al, 2013)

Em 2019, outra revisão sistemática foi realizada para atualizar as informações sobre a influência da PA na saúde sistêmica. Os resultados mostraram uma diferença estatisticamente significativa ao comparar os níveis sistêmicos de proteína C reativa em indivíduos com PA moderada e severa, de acordo com o índice PAI, com os indivíduos do grupo controle, sendo que para PA severa houve uma baixa heterogeneidade entre os estudos e para a análise de PA moderada, nenhuma heterogeneidade esteve presente. Além disso, os resultados mostraram diferenças nos níveis do complexo imune circulatório, óxido nítrico e Interleucina – 6 (IL-6). Através deste estudo, é possível perceber que há fortes evidências de que a PA contribui para a inflamação sistêmica de baixo grau, porém, novos estudos devem ser realizados, com uma metodologia de inclusão e exclusão melhor delimitada e com uma amostra mais relevante da população. (GEORGIOU, 2019). Além disso, a magnitude do aumento do PCR observados no

estudo de Georgiou et al 2019, sugere que há um aumento no risco de doença cardiovascular, pela associação entre a PA e as doenças ateroscleróticas cardíacas.

A sáude bucal da população e a utilização dos serviços em saúde, segundo o modelo proposto por Andersen, depende da utilização dos serviços em saúde, dos fatores que impedem ou favorecem a utilização e a necessidade de cuidado solicitado pelas pessoas. O local de residência é um fator sociodemográfico importante, pois pode alterar a utilização dos serviços em saúde, os comportantos e favorecer as disparidades. (ANDERSEN, 1995)

A literatura documenta bem a diferença entre a zona rural e urbana nos comportamentos relacionados a saúde bucal (ZHOU et al. 2021; VARGAS et al. 2002). Até onde sabemos, não há estudos que utilizem a população rural como amostra para investigação das repercusões sitêmicas da periodontite apical. Portanto, deve-se considerar que as pessoas residentes na zona rural podem diferir-se com relação as pessoas da zona urbana em relação a assitência em saúde bucal, a procura pela mesma, sendo assim, ter uma condição bucal mais precária, o que favorece as doenças bucais infecciosas, tais como a periodontite apical, influenciando na saúde sistemica deste paciente.

Considerando as pesquisas anteriores que relacionam os níveis de PCR a PA, considera-se importante o pleno conhecimento sobre as reais consequências que esse marcador inflamatório pode gerar para a saúde do paciente com alterações endodônticas.

Pesquisas de base populacional apresentam um grande potencial para uma compreensão detalhada tanto da distribuição de diferentes condições que afetam a saúde das mesmas, quanto para o estabelecimento de associações entre diferentes indicadores/fatores de risco para estas condições. Deste modo, o levantamento epidemiológico realizado na área rural de Rosário do Sul, permite que sejam estudadas as possíveis associações entre lesões periapicais e suas repercuções sistêmicas avaliadas neste estudo através dos níveis de Proteína C reativa.

Portanto, este estudo tem como objetivo avaliar a associação entre a presença de periodontite apical e a elevação nos níveis séricos de proteína C-reativa com base em dados obtidos em uma pesquisa epidemiológica de uma amostra representativa

A associação entre lesão periapical e níveis séricos de proteína C-reativa ainda não está consolidada na literatura, pois a maioria deles não utilizaram amostras representativas, não apresentam análises multivariadas e nenhum estudo foi realizado utilizando como amostra uma população rural. No entanto estes estudos anteriores demonstraram uma associação entre presença de lesão periapical e aumento dos marcadores inflamatórios no sangue, como a PCR. Diante disso, nossa hipótese é que a presença de lesão (ões) periapical(is) está associada ao aumento nos níveis séricos de PCR.

2. ARTIGO: ASSOCIATION BETWEEN APICAL PERIODONTITIS AND SERUM C-REACTIVE PROTEIN LEVELS IN A REPRESENTATIVE RURAL SAMPLE FROM SOUTH BRAZIL

Trabalho formatado de acordo com as normas da revista International Endodontic Journal (Anexo C)

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Association between apical periodontitis and serum C-reactive protein levels in a representative rural sample from South Brazil

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ABSTRACT

Objective: The aim of the present cross-sectional study was to determine whether the levels of apical periodontitis (AP) was independently associated with C-reactive protein (CRP) levels in individuals living in a rural area from south Brazil.

Methods: A representative sample obtained from a cross-sectional study that is part of an epidemiological survey carried out in the rural area of southern Brazil of probability (N = 515) was evaluated using periapical whole-mouth radiographs. BP extension was evaluated by the Periapical Index (PAI) and considered as low level (<10% of teeth present with PAI 4 or 5 and PAI 2 or 3) and high level (≥ 10% of PAI 4 or 5). Regression analyzes evaluated associations between AP and CRP levels, adjusting for sex, age, body mass index, glycated hemoglobin, systolic blood pressure e periodontal disease.

Results: In the adjusted model, individuals with high levels of AP showed elevated CRP levels when compared to those with low levels of AP ($p<0.05$). Moreover, individuals in hight level had a 2.3-fold greater chance of having CRP above 5 mg/L than their counterparts (Odds Ratio = 2.30; 95% confidence interval: 1.31 to 4.05).

Conclusion: Therefore, apical periodontits can influence systemic biomarkers, ultimately affecting the patient's general health. High levels of AP were independently associated with higher levels of CRP in individuals living in rural areas, in addition, an association was observed between the extent and severity of apical periodontitis, ie, the burden of endodontic disease in relation to elevation in the levels of inflammatory mediators.

Introduction

Apical periodontitis (AP) is an inflammatory disorder within the periapical tissues of teeth with infected root canal systems, which ultimately leads to the destruction of the apical tissues (Nair, 2004). Such effect is evident as a radiolucency around the roots of the affected tooth and is the consequence of a complex interplay between microorganisms and the activated innate and adaptive immune system of the host, as well as microbial products (Márton & Kiss, 2014). It is estimated that almost half of the adult population worldwide have at least one tooth with AP (Tibúrcio-Machado et al. 2021), which correlates well with an increased global prevalence of untreated caries in the permanent dentition (Bernabe et al., 2020).

There is increasing evidence linking general health and AP, which emphasises the potential importance of oral health on general health (Segura-Egea *et al.* 2015). Albeit perceived as the local destruction of periapical tissues, AP has additional systemic inflammatory ramifications (Georgiou *et al.* 2019).

A systematic review and meta-analysis revealed that AP was associated with increased systemic inflammation, including, for instance, increased C-reactive protein (CRP) levels (Georgiou *et al.* 2019). This specific marker has been reported to be associated with both incidence and progression of cardiovascular conditions, as well as increased mortality rates (Avan et al. 2018). The plausibility of this generalised problem stems from an oral infection, which spreads through the blood system and activates the systemic immune response, ultimately leading to the development of generalised low-grade inflammation.

Sociodemographic indicators, such as the individual's area of residence, can modify behaviors in relation to oral health and be considered indicators of disparity. The literature well documents the difference between rural and urban areas in the oral health-related behaviors of these patients (Zhou et al. 2021; Vargas et al. 2002). However, there are no studies that use the rural population as a sample to investigate the systemic repercussions of apical periodontitis.

The association between periapical lesion and C-reactive protein levels is demonstrated in the literature, however most studies did not use representative samples and many studies only offer bivariate analyzes that were not adjusted for possible confounding factors. Despite this, these previous studies demonstrated an association between the presence of periapical lesion

and the increase of inflammatory markers in the blood, such as CRP. Therefore, our hypothesis is that the presence of periapical lesion(s) leads to an increase in CRP levels.

Therefore, this study aims to assess levels of C-reactive protein in relation to the presence of periapical injury, based on data obtained in an epidemiological survey of a representative sample.

Materials and methods

Sample selection and study design

This cross-sectional observational study belongs to an epidemiological survey carried out between March 2015 and May 2016, in the rural area of the municipality of Rosário do Sul, RS (Ferreira et al. 2019) and abides by the STROBE guidelines (Von Elm et al., 2007). Individuals eligible to participate in the study signed an informed consent form (ICF), and this study was approved by the Research Ethics Committee (REC) of the Federal University of Santa Maria (UFSM), RS, Brazil (CAAE: 37862414.5. 0000.5346). Details on the sampling process have been described elsewhere (Ferreira et al. 2019).

The main outcome considers the analysis of CRP values in relation to the main predictor being apical periodontitis, according to the classification "Periapical index". For this study, individuals aged 18 or over who presented radiography of at least one dental element and CRP measurement were eligible. Edentulous individuals, who did not present clinical and/or radiographic evaluation, or whose radiography was not valid, were conversely excluded.

The power of the current analysis was estimated upon the following parameters: (a) a non-paired design; (b) alpha error of 0.05; and (c) median and interquartile range differences between high level [2.51 (1.23 – 6.82)] and low level [2.33 (1.12 – 4.46)] groups (O'Keefe et al., 2017). The power of the sample reached 97% for the independent variable of interest.

Data collection

Demographic, economic, medical, and behavioural variables were collected through two structured surveys. For the sake of participants' privacy, surveys were applied individually, as interviews, by trained dentists.

The body mass index (BMI) was classified according to the guidelines recommended by the WHO (1998), in which values less than 25 kg/m² are considered healthy individuals; values between 25 and 29.9 kg/m² are considered overweight; and those above 30 kg/m², obesity.

Values referring to systolic blood pressure and glycated haemoglobin were also evaluated. Regarding the medications used, the use of reported antibiotics and anti-inflammatory drugs was considered. Regarding smoking status, the Pack Years classification was used, which is calculated by multiplying the duration by the daily consumption of smoke (Prignot, 1987).

To assess periodontal status, we used the variable related to the mean number of sites with attachment loss greater than 4 mm and the mean of sites with bleeding on probing.

Assessment of radiographic

Four trained dentists performed a periapical survey using the periapical parallelism technique using a 60KvP and 10Ma ProDental X-ray machine, a RVG # 15100 digital sensor, Kodak (Carestream Dental, GA, USA) and a RINN XCP periapical positioner -DS (Dentsplay), following the manufacturer's orders. The images were captured using the Kodak Dental System Imaging programme and processed by software installed on an Acer Aspire 1410 notebook. The radiographic images were analysed using a 20-inch monitor, AOC resolution (Manaus - AM) 1600x900 60Hz, in a dark room, by a single previously trained examiner, and calibrated with 20 periapical radiographies randomly chosen from the same database. This examiner had an inter-examiner Kappa (K) coefficient of 0.8 and an intra-examiner Kappa (K) coefficient of 0.7.

Third molars were not radiographed. From the radiographed teeth, the apical region was evaluated, and later classified by the "The Periapical Index" (PAI) on a scale of 5, on which 1 represents normal periapical structures; 2, small changes in bone structure; 3, changes in bone structure with some mineral loss; 4, periodontitis with a well-defined radiolucent area; and 5, severe periodontitis with exacerbated features. The remaining roots were included in the radiographic testing, and those whose periapical structure was not visible radiographically or without sharpness were excluded. Multirooted teeth had only the root with the highest PAI index considered for classification. After radiographic evaluation, they were divided according to PAI, in which individuals with PAI 1, low level PAI 2 or 3 and 4 or 5 in less than 10% of the teeth present and high level PAI 4 or 5 in more were considered absent. 10% of teeth present.

C-reactive protein evaluation

Blood collection was performed by a nursing technician from an accredited laboratory of the Municipality of Rosário do Sul-RS, in accordance with the screening guidelines from the World Health Organisation (WHO, 2010). Quantitative C-reactive protein levels were measured in blood using the turbidimetry method. The reference value used for CRP evaluation was 5mg/L, observed in the 75% percentile of the sample.

Statistical analysis

Data analysis was performed with the Stata 14.0 software (Stata Corporation; College Station, TX, USA). Descriptive analysis was conducted to assess associations between outcome and predictors. Differences between groups were compared using the chi-square test, Mann-Whitney's U test, Kruskal-Wallis's test followed by Dunn's post hoc test and Student t-test, as appropriate. Thereafter, linear regression was performed to first analyse the associations between variables and natural log-transformed hsCRP values. Later, binary logistic regression was used to estimate the likelihood of having hsCRP levels in the 75th percentile (>5 mg/L), using the counterparts as reference group. This strategy enabled the estimation of odds ratios (ORs) between comparison groups and respective 95% confidence intervals (CIs) adjusted for the effects. The independent variables with a p value < 0.2 in the unadjusted analysis were added to the adjusted model, and a significance level of 5% was adopted to retain the variables in the model, with a backward variable selection.

Results

For the epidemiological survey, 1087 individuals met the criteria for eligibility and, of those, 399 did not participate in the study because they refused to, were unable to attend the examination site, or for other unspecified reasons, in addition to those with incomplete evaluation. Of the 688 individuals who participated in the survey, 515 were included in the study. The excluded participants comprised 71 edentulous individuals, 94 without collection for CRP, and 9 without radiographic examination. (Figure 1)

The means of C-reactive protein results related to categorical variables are described in Table 1. Amongst the variables evaluated, it can be observed that most individuals were male

(50.7%), with a median of 1.75 mg/L of CRP and females (49.3%) with a median of 3.44 mg/L. In relation to BMI, the highest average is found in the >30 kg/m² category (4.01 mg/L). As for the extension of AP, the low-level category was the most frequent (41.7%), in which the median CRP was 2.09 mg/L whilst, in the high-level category, it was 2.51 mg/L. Data regarding gender, BMI, and AP were statistically significant. Most participants reported never having smoked (49.2%) and not to be taking any medication (65.2%).

Table 2 shows the distribution of high-sensitivity C-reactive protein categories according to the characteristics of the participants. Of the 515 individuals included, 387 (75.1%) had CRP values lower than 5 mg/L and 128 (24.9%) individuals had values greater than 5 mg/L. The mean age of individuals with CRP below 5 mg/L and above 5 mg/L was, respectively, 47.76 (±0,79) and 47.48 (± 1,26). Data regarding BMI, systolic blood pressure, glycated haemoglobin, and the extent of apical periodontitis were statistically significant ($p<0.005$).

In the adjusted analysis, the variables sex male (OR = 0.39; 95% CI: 0.25-0.61), BMI from 25 to 29.9 kg/m³ (OR=1.95; 95% CI: 1.04 - 3.67), BMI greater than 30 kg/m³ (OR 4.25; 95% CI: 2.32-7.78) and extension of apical periodontitis (high-level category (OR: 2.30; 95% CI: 1.31) – 4.05) were statistically significant when associated with CRP (see Table 3).

Discussion

This study aimed to assess the alterations in C-reactive protein levels in patients with periapical injuries in a representative sample of the rural population of southern Brazil. The results of this study demonstrated a significant association between AP and high levels of CRP in these individuals, thus confirming our conceptual hypothesis, akin to previous studies (Sirin et al. 2019, Vidal et al. 2016).

The literature well documents the difference between rural and urban areas in oral health-related behaviors (Zhou et al. 2021; Vargas et al. 2002). As far as we know, there were no studies that used the rural population as a sample to investigate the systemic repercussions of apical periodontitis. Therefore, it should be considered that people living in rural areas may differ from people in urban areas in relation to oral health care, the demand for it, thus having a more precarious oral condition, which favors infectious oral diseases, such as apical periodontitis, influencing the systemic health of this patient.

Apical periodontitis was assessed using the PAI scoring system, which aims to standardise and assess the presence and severity of PA with radiographic images (Orstavik et al. 1986) — being widely used in epidemiological studies (Ridao-Sacie et al. al. 2007, Delano et al. 2001, Virtanen et al. 2017). However, despite being a standard in the evaluation, the PAI index considers the individual tooth, without considering the number of affected teeth. In other studies, the assessment between the association of AP with inflammatory markers and systemic diseases used the PAI index without changes, that is, whether AP was present or not, without considering the number of teeth involved and the severity of cases (Vidal et al. 2016, Cotti et al. 2015, Petersen et al. 2014).

In this sense, in addition to evaluating AP using the PAI index, the number of teeth involved in each individual was also considered in order to assess the possible consequences according to the severity of the condition. Existing literature dichotomises the PAI into scores of 1,2 and 3,4 and 5, which may partially reduce the sensitivity of identifying sick individuals, as, in the risk groups, we might have many individuals with PAI 5 or few with PAI 5 and many with PAI 3. Using a criterion of disease extension, we increased the sensitivity because, in addition to the individuals being diagnosed with PAI 3, 4 or 5, this should be identified in more than 10% of the teeth affected. Therefore, in addition to assessing AP using the PAI index, the number of teeth involved in each individual was also considered so as to assess the possible consequences according to the severity of the condition.

The classification according to the number of teeth affected by AP and the relationship with CRP levels was analysed by Sirin et al (2019), who found significantly higher values in patients who were diagnosed with more than one tooth with PAI 3 or 4 or at least 1 teeth with PAI 5 when compared to individuals with one tooth with PAI 3 or 4 or no tooth affected — and the relative risk was twice as high in patients with AP, thus suggesting that CRP levels may significantly increase as the severity of AP increases. These results are similar to what was found in the current study, since patients classified as having more than 10% of teeth present with PAI 4 or 5 had higher levels of CRP when compared to individuals without the periapical alteration.

The relationship of apical periodontitis with inflammatory markers and pro-inflammatory cytokines was also studied by Vidal et al. (2016), who reported high CRP levels in the presence of AP in hypertensive patients. Garrido et al. (2015), histologically investigated extracted teeth with periapical injuries and found higher levels of CRP in the periodontal ligaments of these teeth when compared to healthy teeth. However, in a systematic review

carried out in 2013 by Gomes et al., the association between AP and rise in CRP levels was not established. In a more recent systematic review, however, there was a statistically significant difference when comparing the systemic levels of C-reactive protein in individuals with moderate and severe AP, according to the PAI index, with individuals in the control group (Georgiou et al. 2019).

Recent studies with the aim of assessing the reduction of CRP levels after endodontic treatment in patients with endodontic injuries demonstrate reduction of systemic inflammation when compared to individuals who did not undergo treatment (Bergandi et al. 2019, Poornima et al. 2020). However, in this study, it is not possible to establish a causal relationship.

In the current study, the CRP value in patients with high-risk AP was 2.51 (1.23 – 6.82), which is in agreement with the results by Garrido et al. (2019), who reported median CRP values of 2.5 mg/L for patients with apical periodontitis. However, in patients with PAI 1 — that is, without periapical alterations —, the mean CRP value was 2.83 (1.12 – 4.49), as this is a univariate analysis and did not consider possible confounding variables. In the multivariate analysis, the relationship between CRP and high-level AP showed an independent association with an OR of 2.30. In the low-level category, it was not possible to establish a significant relationship with AP.

In addition, after adjusted analysis, the BMI and gender variables were also significant in the present study. BMI > 30 kg/m2 showed a significant relationship with higher levels of CRP. A study that investigated the association between BMI with periodontal disease and CRP reported a positive association between periodontal disease and CRP, but not with BMI (Grupta et al. 2017). Despite this, in healthy adults, chronic periodontitis and body mass index (BMI) have been associated with increased CRP levels (Slade et al. 2003). Due to the similarities in periodontal and endodontic disease, it is possible that BMI and AP together influence CRP levels.

As this is a cross-sectional study with a large population, it was decided not to exclude from the study those patients with systemic changes and periodontal disease. However, through the multivariate analysis, these variables had no influence on the result, that is, we found an independent association between CRP and AP.

The manifestation of AP can occur in several ways, from asymptomatic with only radiographic signs to major clinical signs of inflammation. This manifestation will depend on the intensity of the aggression (LOPES, SIQUEIRA, 2015). In cases where there are

characteristic clinical manifestations of an acute inflammatory process, such as pain, swelling, intervention will be necessary and usually urgent. However, in asymptomatic cases, even if they also require mandatory intervention, these injuries can go unnoticed and continue to affect the patient's general health (Georgiou et al. 2019, Lopes & Siqueira 2015).

Therefore, according to the results herein obtained, we can consider that apical periodontitis can influence systemic biomarkers, such as CRP, and influence the patient's systemic health. However, more studies with a well-defined design and exclusion factors should be carried out in the future.

Conclusion

High levels of AP were independently associated with higher CRP levels in individuals living in a rural area. Furthermore, the extent and severity of AP was directly proportional to the increase in CRP levels in this population sample. The variables BMI and sex were also associated with increased levels of CRP. Therefore, apical periodontitis can influence systemic biomarkers, ultimately affecting the patient's general health.

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Tables

Table 1: High sensitivity C-reactive protein levels according to categorical variables (N = 515).

Variables	N (%)	hsCRP[a] levels (mg/L)	
		Median (IQR[b])	P
Sex			≤ .001[d]
Female	254 (49.3)	3.44 (1.63 – 6.64)	
Male	261 (50.7)	1.75 (0.84 – 3.14)	
Income[c]			0.681[d]
≤ R$1100	263 (51.1)	2.24 (0.97 – 5.13)	
> R$1100	252 (48.9)	2.37 (1.23 – 4.58)	
Smoking status			0.746[e]
Never smoker	253 (49.2)	2.60 (1.17 – 4.80)	
Light smoker	181 (35.0)	2.31 (1.05 – 4.99)	
Heavy smoker	81 (15.8)	2.08 (1.26 – 5.03)	
Body mass index			≤ .001[e]*
< 25 kg/m^2	148 (28.8)	1.23 (0.54 – 2.42)	
25 to 29.9 kg/m^2	189 (36.6)	2.12 (1.12 – 4.28)	
> 30 kg/m^2	178 (34.6)	4.01 (2.35 – 7.40)	
Medications			0.377[d]
No	336 (65.2)	2.37 (1.10 – 4.69)	
Yes	179 (34.8)	2.39 (1.23 – 5.46)	
Extent of severe apical periodontitis			0.080[e]
Absent (PAI 1 teeth)	195 (37.9)	2.83 (1.12 – 4.49)	
Low level (PAI 2, 3 and < 10% of PAI 4 or 5 teeth)	215 (41.7)	2.09 (1.05 – 4.30)	
High level (≥ 10% of PAI 4 or 5 teeth)	105 (20.4)	2.51 (1.23 – 6.82)	

[a]hsCRP, high-sensitivity C-reactive protein;

[b]IQR, interquartile range;

[c]R$5.35 was approximately equivalent to US$1.00;

[d]Differences between groups analyzed using Mann-Whitney's U test;

[e]Differences among groups analyzed using Kruskal-Wallis's test;

*Significant differences between groups at $p < 0.05$ analyzed using Dunn's post hoc test;

Table 2: Distribution of high-sensitivity C-reactive protein categories according to characteristics of the study participants (N=515).

Variables	hsCRP[a] groups		P
	≤ 5 mg/L	> 5 mg/L	
Number of subjects	387 (75.1)	128 (24.9)	
Demographic variables			
Sex			≤ .001[c]
Female	167 (43.2)	87 (68.0)	
Male	220 (56.8)	41 (32.0)	
Age	47.76 ± 0.79	47.48 ± 1.26	0.854[d]
Socioeconomic variable			
Income[b]			0.244[c]
≤ R$1100	192 (49.5)	71 (56.0)	
> R$1100	195 (50.5)	57 (44.0)	
Behavioural variable			
Smoking status			0.076[c]
Never smoker	191 (49.4)	62 (48.8)	
Light smoker	136 (35.1)	45 (34.7)	
Heavy smoker	60 (15.5)	21 (16.5)	
Medical variables			
Body mass index			≤ .001[c]
< 25 kg/m^2	131 (33.9)	17 (13.4)	
25 to 29.9 kg/m^2	149 (38.5)	40 (30.7)	
> 30 kg/m^2	107 (27.6)	71 (55.9)	
Systolic blood pressure	124.53 ± 1.02	130.02 ± 1.83	0.008[d]
Glycated hemoglobin (Hb1Ac)	5.68 ± 0.03	5.86 ± 0.10	0.016[d]
Medications			0.251[c]
No	258 (66.8)	78 (60.6)	
Yes	129 (33.2)	50 (39.3)	
Oral health-related variables			
Mean PD ≥ 4mm sites (N)	12.40 ± 0.88	11.55 ± 1.72	0.642[d]
Mean BoP sites (N)	45.33 ± 1.35	48.12 ± 2.32	0.302[d]
Extent of severe apical periodontitis			0.004[c]
Absent (PAI 1 teeth)	151 (39.0)	44 (34.4)	
Low level (PAI 2, 3 and < 10% of PAI 4 or 5 teeth)	170 (43.9)	45 (35.1)	
High level (≥ 10% of PAI 4 or 5 teeth)	66 (17.0)	39 (30.5)	

Age, systolic blood pressure, glycated hemoglobin, mean number of sites with PD ≥ 4mm and mean number of BoP sites are presented as mean ± standard error. Categorical variables are presented as number and percentage;
ahsCRP, high-sensitivity C-reactive protein;
bR$5.35 was approximately equivalent to US$1.00;
cDifferences among groups analyzed using Chi-square test;
dDifferences among groups analyzed using t-test;

Table 3: Unadjusted and adjusted associations between extent levels of severe apical periodontitis, other variables of interest and high levels of hsCRP (> 5 mg/L) determined using binary logistic regression.

Variables	Unadjusted model		Final model	
	OR[a] (95% CI[b])	**P**	**OR (95% CI)**	**P**
Demographic variables				
Sex				
Female	Reference		Reference	
Male	0.36 (0.23 – 0.55)	≤ .001	0.39 (0.25 – 0.61)	≤ .001
Age	1.00 (0.99 – 1.01)	0.854	[d]	
Socioeconomic variable				
Income[c]			[d]	
≤ R$1100	Reference			
> R$1100	0.77 (0.51 – 1.15)	0.206		
Behavioural variable				
Smoking status			[d]	
Never smoker	Reference			
Light smoker	1.00 (0.64 – 1.55)	0.988		
Heavy smoker	1.08 (0.61 – 1.91)	0.797		
Medical variables				
Body mass index				
< 25 kg/m^2	Reference		Reference	
25 to 29.9 kg/m^2	2.02 (1.09 – 3.73)	0.026	1.96 (1.04 – 3.67)	0.036
> 30 kg/m^2	5.11 (2.84 – 9.20)	≤ .001	4.25 (2.32 – 7.78)	≤ .001
Systolic blood pressure	1.01 (1.00 – 1.02)	0.009	[e]	
Glycated hemoglobin (Hb1Ac)	1.34 (1.04 – 1.74)	0.026	[e]	
Medications			[d]	
No	Reference			
Yes	1.30 (0.86 – 1.97)	0.210		
Oral health-related variables				
PD ≥ 4mm (N)	1.00 (0.99 – 1.01)	0.642	[d]	
Mean BoP	1.00 (1.00 – 1.01)	0.302	[d]	
Extent of severe apical periodontitis				
Absent (PAI 1 teeth)	Reference		Reference	
Low level (PAI 2, 3 and < 10% of PAI 4 or 5 teeth)	0.91 (0.57 – 1.45)	0.689	0.92 (0.56 – 1.51)	0.744
High level (≥ 10% of PAI 4 or 5 teeth)	2.03 (1.21 – 3.41)	0.008	2.30 (1.31 – 4.05)	0.004

[a]OR, odds ratio;

[b]CI, confidence interval;

[c]R$5.35 was approximately equivalent to US$1.00;

[d]Variables not included in the adjustment (p > 0.2);

[e]Variables not retained after the adjustment (p > 0.05);

Suplementary material

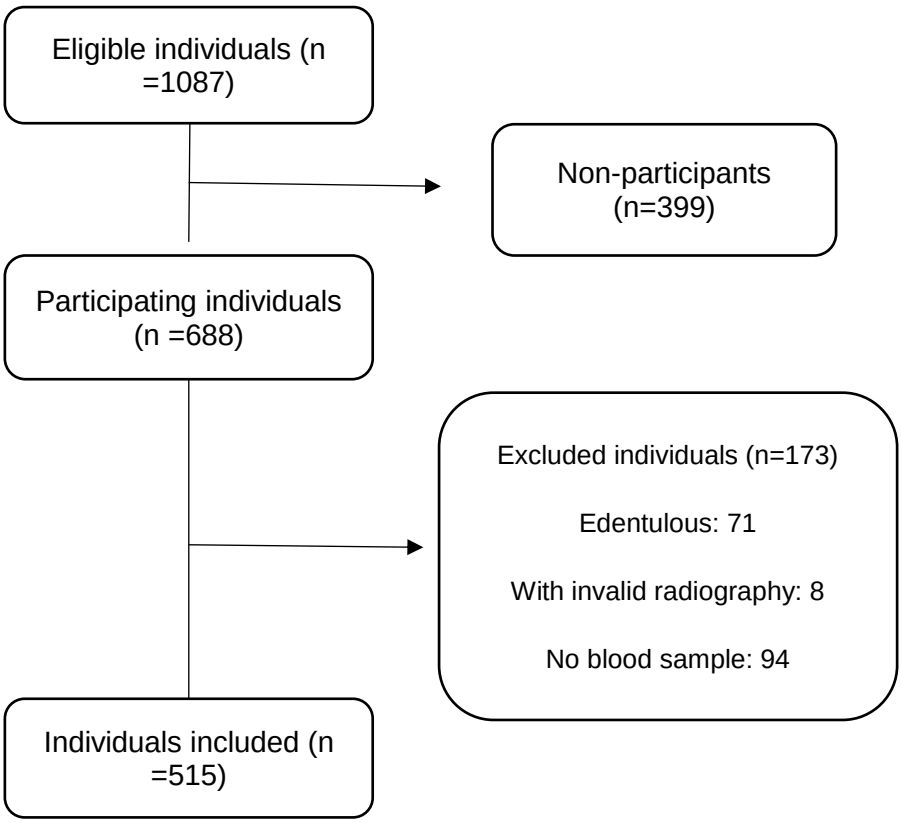
Table 4: Unadjusted and adjusted associations between extent levels of severe apical periodontitis, other variables of interest and log_e- transformed C-reactive protein levels determined using linear regression.

Variables	Unadjusted model		Final model	
	ß[a] (SE[b])	p	ß (SE)	P
Demographic variables				
Sex				
Female	Reference		Reference	
Male	-0.0631 (0.0122)	≤ .001	-0.501 (0.0121)	≤ .001
Age	-0.0001 (0.0004)	0.713	[d]	
Socioeconomic variable				
Income[c]			[d]	
≤ R$1100	Reference			
> R$1100	-0.0001 (0.0126)	0.996		
Behavioural variable				
Smoking status			[d]	
Never smoker	Reference			
Light smoker	0.0035 (0.0138)	0.800		
Heavy smoker	-0.0060 (0.0182)	0.743		
Medical variables				
Body mass index				
< 25 kg/m^2	Reference		Reference	
25 to 29.9 kg/m^2	0.0417 (0.0150)	0.006	0.0378 (0.0147)	0.010
> 30 kg/m^2	0.1059 (0.0151)	≤ .001	0.0918 (0.0152)	≤ .001
Systolic blood pressure	0.0007 (0.0003)	0.034	[e]	
Glycated hemoglobin (Hb1Ac)	0.0120 (0.0085)	0.158	[e]	
Medications				
No	Reference			
Yes	0.0113 (0.0133)	0.397	[d]	
Oral health-related variables				
PD ≥ 4mm (N)	-0.0005 (0.0003)	0.177	[e]	
Mean BoP	0.0002 (0.0002)	0.304	[d]	
Extent of severe apical periodontitis				
Absent (PAI 1 teeth)	Reference		Reference	
Low level (PAI 2, 3 and < 10% of PAI 4 or 5 teeth)	-0.0105 (0.0140)	0.455	-0.0085 (0.0132)	0.518
High level (≥ 10% of PAI 4 or 5 teeth)	0.0271 (0.0172)	0.045	0.0293 (0.0163)	0.033

[a]ß, regression coefficient;
[b]SE, standard error;
[c]R$5.35 was approximately equivalent to US$1.00;
[d]Variables not included in the adjustment (p > 0.2);
[e]Variables not retained after the adjustment (p > 0.05);

Figures

Figure 1. Inclusion and exclusion factors flowchart



3. CONSIDERAÇÕES FINAIS

O presente estudo avaliou as alterações sistêmicas causadas por lesões periapicais observadas através da proteína C- reativa, em uma amostra obtida a partir de um levantamento epidemiológico em uma população rural na zona sul do Brasil. Estudos recentes demonstram que as infecções de origem endodôntica podem influenciar os níveis de mediadores inflamatórios sistêmicos e afetar a saúde geral do paciente.

A presença da PA, neste estudo, influenciou independentemente os níveis de proteína C- reativa. Além disso, a extensão e severidade da PA foram diretamente proporcionais ao aumento nos níveis de PCR. As variáveis IMC e sexo também foram estatisticamente significantes neste estudo. Portanto os presentes achados suportam a hipótese de que a presença de PA pode elevar os níveis PCR.

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ZHOU, Yuqiao et al. Oral health and related risk indicators in north-central Appalachia differ by rurality. **Community Dentistry and Oral Epidemiology**, v. 49, n. 5, p. 427-436, 2021.

APÊNDICE A – TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO PARA PARTICIPANTES MAIORES DE 18 ANOS

Universidade Federal de Santa Maria
Centro de Ciências da Saúde
Programa de Pós-Graduação em Ciências Odontológicas

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do projeto: Levantamento epidemiológico na área rural de Rosário do Sul-RS
Pesquisador responsável: Carlos Heitor Cunha Moreira
Instituição/Departamento: Universidade Federal de Santa Maria / Programa de Pós-Graduação em Ciências Odontológicas.
Telefone para contato (inclusive a cobrar): (55) 9106-4673
Pesquisadores participantes: Jociana Boligon e Ticiane de Góes Mário.
Telefone para contato (inclusive a cobrar): (55) 9978-0866 e (55) 9903-5101

- Você está sendo convidado(a) para participar, como voluntário, em uma pesquisa. Você precisa decidir se quer participar ou não. Por favor, não se apresse em tomar a decisão. Leia cuidadosamente o que se segue e pergunte ao responsável pelo estudo qualquer dúvida que você tiver. Após ser esclarecido sobre as informações a seguir, no caso de aceitar fazer parte do estudo, assine ao final deste documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. Em caso de recusa você não será penalizado de forma alguma.
- Essa pesquisa justifica-se pela necessidade de conhecimento das condições periodontais e saúde geral de uma população que, pela localização geográfica, extensão territorial, diversidades socioeconômica e cultural, tem dificuldade de acesso à assistência médica e odontológica integral.
- A sua participação nesse estudo será no sentido de permitir a avaliação da sua boca, de suas medidas corporais e de responder a alguns questionários. Serão anotados dados sobre a quantidade de dentes perdidos, restaurados, obturados e cariados; a presença de placa (tecido amolecido amarelo-esbranquiçado) e cálculo dentário (tecido duro de cor mais escura) formados sobre seus dentes; a ocorrência de sangramento ou pus na sua gengiva e medidas de perda de osso ao redor dos seus dentes, quando encostamos um instrumento odontológico (sonda periodontal milimetrada) entre essas duas estruturas e se há alteração na gengiva após esta ser corada com uma substância inofensiva à sua saúde. Você responderá a questionários, de rápida execução, sobre consultas ao dentista, presença de doenças ou alterações em seu organismo, uso de remédios, hábitos alimentares e comportamentais, nível de educação, renda familiar e qualidade de vida. Seu peso e sua altura serão medidos para análise do seu Índice de Massa Corporal. Também mediremos a circunferência da sua cintura e verificaremos sua pressão arterial, e um técnico em enfermagem capacitado (de um laboratório conveniado da prefeitura do município) coletará amostras de sangue para melhor avaliarmos sua saúde geral.
- Você poderá se sentir cansado e ter algum desconforto nos exames em que um instrumento odontológico é passado entre sua gengiva e seus dentes, além de haver um risco mínimo de se machucar com o instrumento caso ocorra um movimento brusco de sua parte ou do examinador. Após os exames você poderá ficar com dor leve em sua gengiva. Desconforto também poderá ser sentido durante a coleta de material sanguíneo. Além disso, você poderá se sentir constrangido ou cansado em responder as questões dos questionários ou, ainda durante medição do seu peso e altura. Caso haja dano odontológico com a pesquisa você terá direito a assistência odontológica gratuita garantida pelos pesquisadores.
- O benefício direto a você, participante, será um relatório odontológico detalhado sobre a condição de sua boca e, se necessário, encaminhamento para tratamento odontológico no Serviço de

Saúde Municipal ou nas Clínicas Odontológicas da Universidade Federal de Santa Maria e uma avaliação complementar do seu estado de saúde geral.

❖ Você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de eventuais dúvidas em qualquer etapa do estudo. É garantido o livre acesso a todas as informações e, sendo de seu interesse, você será mantido atualizado sobre os resultados finais da pesquisa após a publicação da mesma.

❖ Se você concordar em participar do estudo, seu nome e identidade serão mantidos em sigilo. A menos que requerido por lei ou por sua solicitação, somente a equipe do estudo e o Comitê de Ética terão acesso a suas informações. As informações do estudo serão divulgadas apenas em eventos ou publicações científicas sem identificação dos voluntários. As fichas clínicas e os questionários, após analisados, ficarão guardados na Clínica de Periodontia da UFSM Santa Maria/RS. (Antigo Prédio da Reitoria, Rua Marechal Floriano Peixoto, número 1184, 7º andar, sala 710) por 5 anos, a fim de possibilitar esclarecimentos posteriores ao término do estudo, conforme nova resolução do CNS 466/12, e, depois, imediatamente destruídos por incineração. Exames de sangue serão fornecidos ao paciente, nós ficaremos com uma cópia do mesmo, que será armazenada como descrito acima.

❖ Você pode se recusar a participar do estudo, ou retirar seu consentimento e sair da pesquisa a qualquer momento, mesmo durante o exame, sem precisar justificar.

Eu, _____, de nacionalidade _____, com _____ anos de idade, estado civil _____, profissão _____, residente em _____, RG nº _____, abaixo assinado, concordo em participar do estudo como sujeito. Fui suficientemente informado (a) a respeito das informações que li ou que foram lidas para mim, descrevendo o estudo **"Levantamento epidemiológico na área rural de Rosário do Sul-RS".** Eu discuti com a pesquisadora _____ sobre a minha decisão em participar nesse estudo. Ficaram claros para mim quais são os propósitos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Estou totalmente ciente de que não há nenhum valor econômico, a receber ou pagar, por minha participação. Ficou claro também que minha participação é isenta de despesas. Concordo voluntariamente em participar deste estudo e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo, sem penalidades ou prejuízo.

Rosário do Sul, _____ de _____ de 201__.

_____

Nome e Assinatura do sujeito

Declaro que obtive de forma apropriada e voluntária o Consentimento Livre e Esclarecido deste sujeito de pesquisa ou representante legal para a participação neste estudo:

_____

Nome e assinatura do pesquisador responsável

_____

Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato: Comitê de Ética em Pesquisa – UFSM - Cidade Universitária - Bairro Camobi, Av. Roraima, nº1000 - CEP: 97.105.900 Santa Maria – RS. Telefone: (55) 3220-9362 – Fax: (55)3220-8009 Email: comiteeticapesquisa@smail.ufsm.br. Web: www.ufsm.br/cep

ANEXO A – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA NO ANO DE 2014

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: LEVANTAMENTO EPIDEMIOLÓGICO NA ÁREA RURAL DE ROSÁRIO DO SUL/RS
Pesquisador: CARLOS HEITOR CUNHA MOREIRA
Área Temática:
Versão: 1
CAAE: 37862414.5.0000.5346
Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 869.323
Data da Relatoria: 10/11/2014

Apresentação do Projeto:
Doenças periodontais compreendem condições infecciosas e inflamatórias resultantes da interação entre biofilme bacteriano e resposta do hospedeiro. Essa relação é modulada por uma variedade de fatores, dentre eles, diabetes e fumo, capazes de alterar o início e a progressão dessas afecções. A doença periodontal também pode acarretar alterações sistêmicas, como na doença cardiovascular e no controle da glicemia, e comprometimento funcional e estético. O entendimento de uma pequena quantidade de fatores de risco pode ter potencial impacto no encargo de muitas doenças, com custo reduzido e maior eficiência e efetividade que abordagens específicas para cada condição isolada. Assim, esse projeto objetiva avaliar condições bucais, parâmetros inflamatórios e microbiológicos associados, indicadores e fatores de risco às doenças periodontais, impacto desses parâmetros na qualidade de vida, além de questões relacionadas à saúde geral, como obesidade, diabetes e hipertensão, na zona rural de Rosário do Sul - RS.

Realizaremos um censo das crianças de 10 a 14 anos, para avaliação de cárie e fluorose. E uma amostra representativa dos indivíduos, maiores de 15 anos, residentes na área rural desse município (N= 828) receberá exame bucal completo (periodonto, dentes, mucosas, saliva e análise microbiológica de biofilme), avaliações antropométricas (pressão

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Bairro: Camobi **CEP:** 97.105-970
UF: RS **Município:** SANTA MARIA
Telefone: (55)3220-9362 **E-mail:** cep.ufsm@gmail.com

arterial, peso, altura, circunferência da cintura) e exames sanguíneos (hemograma completo, hemoglobina glicada, proteína C-reativa ultrassensível e continina plasmática).
Adicionalmente, os moradores que aceitarem participar do estudo, mediante a assinatura de termo de consentimento livre e esclarecido, responderão a questionários sobre qualidade de vida, características médicas e sociodemográficas e hábitos de higiene bucal.

Esperamos que, através do conhecimento gerado após a análise dos resultados desse projeto, medidas de controle e/ou erradicação dos problemas encontrados possam ser adotadas, visando melhorias na saúde dos indivíduos dessa área. Caso essas estratégias sejam implementadas, avaliações posteriores poderão ser realizadas a fim de verificar a efetividade das mesmas. Além disso, com a obtenção de resultados positivos/benéficos, há a possibilidade de extensão para outras populações, na tentativa de melhorar as condições globais de saúde.

Objetivo da Pesquisa:
Objetivo geral: realizar um levantamento epidemiológico em uma amostra representativa da população rural de Rosário do Sul/ RS.

Objetivos específicos
Avaliar a condição periodontal (prevalência, extensão e gravidade de doença) dessa população;
Buscar associações entre condição periodontal e parâmetros inflamatórios e icrobiológicos;
Avaliar a presença de fatores de risco (fumo e diabetes) para as doenças periodontais;
Verificar possíveis indicadores de risco para doença periodontal;
Investigar o impacto da utilização de protocolos de exame parciais em comparação com exames de toda a boca em prevalência, gravidade e extensão de doença periodontal;
Avaliar prevalência, extensão e gravidade de recessão gengival (RG);
Avaliar a associação de potenciais indicadores de risco com a ocorrência de RG;
Avaliar prevalência, extensão e gravidade de abrasão gengival (AG);
Avaliar a associação de potenciais indicadores de risco com a ocorrência de AG;
Verificar a associação entre AG e RG, identificando se o aumento na prevalência de AG pode gerar aumento na prevalência de RG;
Verificar a associação entre fatores demográficos (sexo, renda, idade e raça), comportamentais (fumo, presença de cálculo...) e as condições de abrasão e recessão gengivais encontradas;

Avaliar o impacto da periodontite como condição clínica preditora de uma pior qualidade de vida relacionada à saúde bucal (OHRQoL);

Investigar as condições clínicas associadas a uma pior OHRQoL;

Avaliar a correlação entre dois instrumentos sócio-dentais, OHIP-14 e GOHAI, para avaliação da OHRQoL;

Avaliar o efeito da avaliação periodontal em boca reduzida realizada por meio de diferentes protocolos parciais nas medidas de associação com a OHRQoL.

Avaliar a condição cariológica das crianças e jovens com idades compreendidas entre 10 e 14 anos;

Buscar associação entre a presença de lesões cariosas ativas e o grau eruptivo dos segundos molares permanentes;

Avaliar os indicadores de risco para cárie dentária;

Avaliar a presença de fluorose dentária.

Avaliação dos Riscos e Benefícios:

Previstos de modo suficiente.

Comentários e Considerações sobre a Pesquisa:

.

Considerações sobre os Termos de apresentação obrigatória:

Termos apresentados.

Recomendações:

Veja no site do CEP - http://coral.ufsm.br/cep - SITE NOVO - na aba "orientações gerais", modelos e orientações para apresentação dos documentos. Acompanhe as orientações disponíveis, evite pendências e agilize a tramitação do seu projeto.

Conclusões ou Pendências e Lista de Inadequações:

.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

SANTA MARIA, 12 de Novembro de 2014

Assinado por:
CLAUDEMIR DE QUADROS
(Coordenador)

Endereço: Av. Roraima, 1000 - prédio da Reitoria - 2º andar
Bairro: Camobi **CEP:** 97.105-970
UF: RS **Município:** SANTA MARIA
Telefone: (55)3220-9362 **E-mail:** cep.ufsm@gmail.com

ANEXO B – CARTA DE APROVAÇÃO DO COMITÊ DE ÉTICA EM PESQUISA NO ANO DE 2015

UNIVERSIDADE FEDERAL DE SANTA MARIA/ PRÓ-REITORIA DE PÓS-GRADUAÇÃO E

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: LEVANTAMENTO EPIDEMIOLÓGICO NA ÁREA RURAL DE ROSÁRIO DO SUL/RS
Pesquisador: CARLOS HEITOR CUNHA MOREIRA
Área Temática:
Versão: 2
CAAE: 37862414.5.0000.5346
Instituição Proponente: Universidade Federal de Santa Maria/ Pró-Reitoria de Pós-Graduação e
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 979.743
Data da Relatoria: 10/03/2015

Apresentação do Projeto:
Doenças periodontais compreendem condições infecciosas e inflamatórias resultantes da interação entre biofilme bacteriano e resposta do hospedeiro. Essa relação é modulada
por uma variedade de fatores, dentre eles, diabetes e fumo, capazes de alterar o início e a progressão dessas afecções. A doença periodontal também pode acarretar alterações
sistêmicas, como na doença cardiovascular e no controle da glicemia, e comprometimento
funcional e estético. O entendimento de uma pequena quantidade de fatores de risco pode ter potencial impacto no encargo de muitas doenças, com custo reduzido e maior eficiência e efetividade que abordagens específicas para cada condição isolada. Assim, esse projeto
objetiva avaliar condições bucais, parâmetros inflamatórios e microbiológicos associados,
indicadores e fatores de risco às doenças periodontais, impacto desses parâmetros na
qualidade de vida, além de questões relacionadas à saúde geral, como obesidade, diabetes
e hipertensão, na zona rural de Rosário do Sul - RS.

Realizaremos um censo das crianças de 10 a 14 anos, para avaliação de cárie e fluorose. E uma amostra representativa dos indivíduos, maiores de 15 anos, residentes na área rural desse município (N= 828) receberá exame bucal completo (periodonto, dentes, mucosas, saliva e análise microbiológica de biofilme), avaliações antropométricas (pressão arterial, peso, altura,

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Bairro: Camobi **CEP:** 97.105-970
UF: RS **Município:** SANTA MARIA
Telefone: (55)3220-9362 **E-mail:** cep.ufsm@gmail.com

circunferência da cintura) e exames sanguíneos (hemograma completo, hemoglobina glicada, proteína C-reativa ultrassensível e continina plasmática).
Adicionalmente, os moradores que aceitarem participar do estudo, mediante a assinatura de termo de consentimento livre e esclarecido, responderão a questionários sobre qualidade de vida, características médicas e sociodemográficas e hábitos de higiene bucal.

Esperamos que, através do conhecimento gerado após a análise dos resultados desse projeto, medidas de controle e/ou erradicação dos problemas encontrados possam ser adotadas, visando melhorias na saúde dos indivíduos dessa área. Caso essas estratégias sejam implementadas, avaliações posteriores poderão ser realizadas a fim de verificar a efetividade das mesmas. Além disso, com a obtenção de resultados positivos/benéficos, há a possibilidade de extensão para outras populações, na tentativa de melhorar as condições globais de saúde.

Objetivo da Pesquisa:
Objetivo geral: realizar um levantamento epidemiológico em uma amostra representativa da população rural de Rosário do Sul/ RS.

Objetivos específicos
- Avaliar a condição periodontal (prevalência, extensão e gravidade de doença) dessa população;
- Buscar associações entre condição periodontal e parâmetros inflamatórios e icrobiológicos;
- Avaliar a presença de fatores de risco (fumo e diabetes) para as doenças periodontais;
- Verificar possíveis indicadores de risco para doença periodontal;
- Investigar o impacto da utilização de protocolos de exame parciais em comparação com exames de toda a boca em prevalência, gravidade e extensão de doença periodontal;
- Avaliar prevalência, extensão e gravidade de recessão gengival (RG);
- Avaliar a associação de potenciais indicadores de risco com a ocorrência de RG;
- Avaliar prevalência, extensão e gravidade de abrasão gengival (AG);
- Avaliar a associação de potenciais indicadores de risco com a ocorrência de AG;
- Verificar a associação entre AG e RG, identificando se o aumento na prevalência de AG pode gerar aumento na prevalência de RG;
- Verificar a associação entre fatores demográficos (sexo, renda, idade e raça), comportamentais (fumo, presença de cálculo...) e as condições de abrasão e recessão gengivais encontradas;

- Avaliar o impacto da periodontite como condição clínica preditora de uma pior qualidade de vida relacionada à saúde bucal (OHRQoL);
- Investigar as condições clínicas associadas a uma pior OHRQoL;
- Avaliar a correlação entre dois instrumentos sócio-dentais, OHIP-14 e GOHAI, para avaliação da OHRQoL;
- Avaliar o efeito da avaliação periodontal em boca reduzida realizada por meio de diferentes protocolos parciais nas medidas de associação com a OHRQoL.
- Avaliar a condição cariológica das crianças e jovens com idades compreendidas entre 10 e 14 anos;
- Buscar associação entre a presença de lesões cariosas ativas e o grau eruptivo dos segundos molares permanentes;
- Avaliar os indicadores de risco para cárie dentária;
- Avaliar a presença de fluorose dentária.

Avaliação dos Riscos e Benefícios:
Previstos de modo suficiente.

Comentários e Considerações sobre a Pesquisa:
.

Considerações sobre os Termos de apresentação obrigatória:
Apresentados de modo suficiente.

Recomendações:
Veja no site do CEP - http://w3.ufsm.br/nucleodecomites/index.php/cep - na aba "orientações gerais", modelos e orientações para apresentação dos documentos. Acompanhe as orientações disponíveis, evite pendências e agilize a tramitação do seu projeto.

Conclusões ou Pendências e Lista de Inadequações:
.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

SANTA MARIA, 10 de Março de 2015

Assinado por:
CLAUDEMIR DE QUADROS
(Coordenador)

ANEXO C – NORMAS DE PUBLICAÇÃO DO PERIÓDICO INTERNATIONAL ENDODONTIC JOURNAL

Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

Useful Websites: Submission Site, Articles published in International Endodontic Journal, Author Services, Wiley's Ethical Guidelines, Guidelines for Figures The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted. Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in International Endodontic Journal. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

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

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

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

Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used. When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance

with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/ . Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

2.4 Systematic Reviews

Systematic reviews should be reported using the PRISMA guidelines available at http://prisma-statement.org/. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

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

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author’s objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal. The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/). It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form,

and to upload all forms individually (do not combine the forms into one file) together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.7 Appeal of Decision

The decision on a paper is final and cannot be appealed.

2.8 Permissions

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

2.8 Copyright Assignment

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• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej

• Log-in, or if you are a new user, click on 'register here'.

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Manuscript that do not conform to the general aims and scope of the journal will be 31 returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor. International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author

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Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Mini Review Articles: are accepted to address current evidence on well-defined clinical, research or methodological topics. All are refereed by experts in the field who are asked to comment on timeliness, general interest, balanced treatment of controversies, and scientific rigor. A clear research question, search strategy and balanced synthesis of the evidence is expected. Manuscripts are limited in terms of word-length and number of figures.

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5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to International Endodontic Journal should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

• Aim: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• Methodology: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• Results: Give the main results of the study, including the outcome of any statistical analysis.

• Conclusions: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.

Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

Abstract for Case Reports should be no more than 250 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• Summary: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• Key learning points: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

• Aim: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• Methodology: Describe the methods adopted.

• Results: Give the main results of the study.

• Conclusions: State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

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

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA

regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures. 35

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Mini Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should briefly introduce the subject matter and justify the need and timeliness of the literature review. The Review section should be divided into logical sub-sections to enhance readability and understanding and may be supported by up to 5 tables and figures. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The Conclusions section should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends should not normally exceed 4000 words.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images, radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

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It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum. We recommend the use of a tool such as EndNote or Reference Manager for reference management and

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In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by et al. is sufficient, e.g. (Tobias et al. 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias et al. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form. (i) Names and initials of up to six authors. When there are seven or more, list the first three and add et al. (ii)Year of publication in parentheses (iii) Full title of paper followed by a full stop (.) (iv) Title of journal in full (in italics) (v) Volume number (bold) followed by a comma (,) (vi) First and last pages Examples of correct forms of reference follow:

Standard journal article
Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. International Endodontic Journal 24, 8-14.
Corporate author
British Endodontic Society (1983) Guidelines for root canal treatment. International Endodontic Journal 16, 192-5.
Journal supplement
Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). Blood 54 (Suppl. 1), 26a.
Books and other monographs
Personal author(s)
Gutmann J, Harrison JW (1991) Surgical Endodontics, 1st edn Boston, MA, USA: Blackwell Scientific Publications.
Chapter in a book
Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. Endodontics in Clinical Practice, 3rd edn; pp. 186-223. London, UK: Butterworth.
Published proceedings paper
DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.
Agency publication
Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8
Dissertation or thesis
Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs
Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text. Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003]

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Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.
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Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.
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7 Guidelines for reporting of DNA microarray data

The International Endodontic Journal gives authors notice that, with effect from 1st January 2011, submission to the International Endodontic Journal requires the reporting of microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available athttp://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the

reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.