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BIOMARCADORES DA HOMEOSTASE REDOX EM SALIVA E ALTERAÇÕES NUCLEARES NO EPITÉLIO DA MUCOSA JUGAL DE INDIVÍDUOS COM DIABETES MELLITUS TIPO 2 E PERIODONTITE

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2023

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Odontologia (Área de Concentração: Periodontia) da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro como parte dos requisitos para a obtenção do Título de Doutor em Odontologia (Área de Concentração: Periodontia).

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Walder Jansen de Mello Lobão

Biomarcadores da homeostase redox em saliva e alterações nucleares no epitélio da mucosa jugal de indivíduos com diabetes mellitus tipo 2 e periodontite

Tese de Doutorado submetida ao Programa de Pós-Graduação em Odontologia (Periodontia), Faculdade de Odontologia, da Universidade Federal do Rio de Janeiro, como parte dos requisitos necessários à obtenção do Grau de Doutor em Odontologia (Periodontia).

Aprovado em ____________

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Prof. Dr. José Eduardo Batista

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Profa. Dra. Lucianne Cople Maia

Este trabalho é dedicado aos meus pais, que Deus os chamou para contemplar a vida eterna ao Seu lado, Zárah Jansen e Walter Lobão. Sei que lá de cima intercedem pelo meu bem, pois sempre apoiaram todos os meus sonhos e me amaram incondicionalmente. Saudades eternas!

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"[...] Eu chuto pra longe toda a má vibração
Eu sou mais que vencedor
Nada nessa vida é impossível pra mim
Eu nasci pra conquistar
Nada nessa vida é impossível pra mim
Nada Nada nessa vida é impossível pra mim
Impossível é uma palavra muito grande que
gente pequena usa pra tentar nos oprimir [...]"

Charlie Brown Jr

RESUMO

BIOMARCADORES DA HOMEOSTASE REDOX EM SALIVA E ALTERAÇÕES NUCLEARES NO EPITÉLIO DA MUCOSA JUGAL DE INDIVÍDUOS COM DIABETES MELLITUS TIPO 2 E PERIODONTITE

Walder Jansen de Mello Lobão

Resumo da Tese de Doutorado submetida ao Programa de Pós-graduação em Odontologia, Centro de Ciências da Saúde, da Universidade Federal do Rio de Janeiro - UFRJ, como parte dos requisitos necessários à obtenção do título de Doutor em Odontologia com área de concentração em Periodontia.

O presente estudo é composto por dois capítulos, sendo o primeiro um artigo de revisão sistemática que abordou as evidências científicas para a diferença de biomarcadores do estresse oxidativo em indivíduos com diabetes mellitus tipo 2 com e sem periodontite. Foram incluídos 9 estudos na análise final, pesquisados através das seguintes bases de dados: PubMed, Scopus, Embase, Web of Science, Cochrane Library, Biblioteca Virtual da Saúde e por outras fontes. Os estudos relataram elevadas concentrações de agentes oxidantes e baixos níveis de antioxidantes em indivíduos com diabetes mellitus tipo 2 e periodontite quando comparados aos indivíduos sem periodontite. Considerando os poucos estudos encontrados, as falhas metodológicas, poucos marcadores estudados e ausência de homogeneidade na avaliação dos marcadores do balanço redox, bem como, a baixíssima certeza da evidência entre os estudos incluídos nesta revisão sistemática, não foi possível determinar se há ou não diferenças nos níveis de estresse oxidativo em indivíduos com diabetes mellitus tipo 2 associado ou não à periodontite e, portanto, estudos observacionais prospectivos e de intervenção são recomendados. O segundo capítulo é um artigo de pesquisa que retrata a periodontite e o diabetes mellitus tipo 2 como doenças com características de inflamação crônica e com estresse oxidativo permanentemente elevado, o que afeta o padrão de funcionamento do sistema imunológico e resulta em danos a importantes macromoléculas biológicas. Trata-se de um estudo observacional, que objetivou avaliar biomarcadores da homeostase redox em

saliva e a presença de alterações nucleares em pacientes com periodontite com ou sem diabetes tipo 2 e em indivíduos periodontalmente e sistemicamente saudáveis. Um total de 60 participantes foram divididos igualmente em três grupos: diabetes tipo 2 com periodontite (DPE); sem diabetes com periodontite (PE); saudáveis sem doença periodontal (HC). Após as medições clínicas periodontais, as células epiteliais da mucosa jugal e a amostra de saliva foram coletadas. O dano ao DNA foi determinado pela contagem de micronúcleos e anormalidades nucleares em células epiteliais. Os níveis de estresse oxidativo foram determinados por glutationa reduzida (GSH), ácido úrico (UA), capacidade antioxidante total (TAC), substâncias reativas ao ácido tiobarbitúrico (TBARs) e proteínas totais. Os dados numéricos foram testados pelos testes de Mann-Whitney e Kruskal-Wallis, as variáveis numéricas pelo teste do qui-quadrado e as correlações pelo coeficiente de Spearman, regressões linear e logística foram realizadas, adotando o nível de significância de 5%. As frequências de micronúcleos, cariorrexia, cromatina condensada e células picnóticas, e dos biomarcadores GSH e UA foram significativamente maiores no grupo DPE seguido dos grupos PE e HC ($p<0,05$). Glicemia em jejum, hemoglobina glicada, frequência de micronúcleos (MN), anormalidades nucleares (NA - cariorrexia, cromatina condensada e células picnóticas), GSH e UA apresentaram de leve a moderada correlação positiva com os parâmetros de progressão da periodontite ($p<0,05$). A análise de regressão linear mostrou que GSH categorizada teve correlação com sangramento gengival ($p = 0,002$) e TBARs ($p = 0,020$) e UA com sangramento à sondagem ($p = 0,001$) e TAC ($p = 0,001$). A análise de regressão logística mostrou que os níveis categorizados de GSH tiveram correlação com sangramento à sondagem (OR = 1,121 [95% CI, 1,025-1,225]) e supuração (OR = 0,155 [95% CI, 0,029-0,838]). Portanto, as correlações positivas entre os parâmetros periodontais, MN, NA (cromatina condensada, células cariorréticas e picnóticas), e os parâmetros redox na saliva (GSH e UA) refletem piores condições periodontais e dos parâmetros do diabetes mellitus tipo 2. Os biomarcadores na saliva (GSH e UA) apresentaram um importante papel na detecção de alteração no funcionamento do balanço redox e dos danos nucleares em células epiteliais da mucosa jugal.

Palavras-chave: estresse oxidativo, dano ao DNA, periodontite, diabetes mellitus tipo 2.

ABSTRACT

OXIDATIVE STRESS BIOMARKERS IN SALIVA AND NUCLEAR CHANGES IN EPITHELIAL CELLS OF INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS AND PERIODONTITIS

Walder Jansen de Mello Lobão

Abstract da Tese de Doutorado submetida ao Programa de Pós-graduação em Odontologia, Centro de Ciências da Saúde, da Universidade Federal do Rio de Janeiro - UFRJ, como parte dos requisitos necessários à obtenção do título de Doutor em Odontologia com área de concentração em Periodontia.

This study consists of two chapters, the first is a systematic review article which addressed the scientific evidence for the difference of oxidative stress biomarkers in individuals with type 2 diabetes mellitus with and without periodontitis. Nine studies were included in the final analysis, searched through the following databases: PubMed, Scopus, Embase, Web of Science, Cochrane Library, Virtual Health Library and other sources. The studies reported high concentrations of oxidizing agents and low antioxidants levels in individuals with type 2 diabetes mellitus and periodontitis when compared to with no periodontitis. Considering the few studies found, the methodological flaws, few markers studied and absence homogeneity in the evaluation of redox balance markers, as well as, the very low certainty of the evidence among included studies in this systematic review, it was not possible to determine whether there are or not differences in the oxidative stress levels in individuals with type 2 diabetes mellitus associated or no with periodontitis and further prospective observational and interventional studies are recommended. The second chapter is a research article that describe the periodontitis and type 2 diabetes mellitus as diseases with features of chronic inflammation and with oxidative stress permanently elevated which affects the pattern of functioning of the immune system and results in damage to important biological macromolecules. This is an observational study, which aimed to evaluate redox homeostasis biomarkers in saliva and the presence of nuclear changes in patients with periodontitis with and without type 2 diabetes and periodontally and systemically healthy individuals. A total of 60

participants were allotted into three groups equally: type 2 diabetes with periodontitis (DPE); non-diabetes with periodontitis (PE); healthy without periodontal disease (HC). After periodontal measurements, cheek epithelial cells and saliva samples were collected. DNA damage was determined by counting micronucleus and nuclear abnormalities in epithelial cells. Oxidative stress levels were determined by reduced glutathione (GSH), uric acid (UA), total antioxidant capacity, thiobarbituric acid reactive substances, and total proteins. Numeric data were tested by Mann-Whitney and Kruskal-Whallis tests, qualitative variables by chi-square test and correlations by the spearman coeficiente, and linear and logistic regression were performed, adopting the significance level of 5%. The frequencies of micronucleus (MN), karyorrhectic, condensed chromatin, pyknotic cells, GSH and UA were significantly higher in DPE group followed PE and HC groups ($p<0.05$). Fasting blood glucose, glycated hemoglobin, MN and nuclear abnormalities (NA) frequencies (karyorrhectic, condensed chromatin and pyknotic cells), GSH and UA showed a positive mild to moderate correlation with periodontitis progression parameters ($p<0.05$). Linear regression analysis showed that categorized GSH had correlation with gingival bleeding ($p = 0.002$) and TBARs ($p = 0.020$) and UA with bleeding on probing ($p = 0.001$) and TAC ($p = 0.001$). Logistic regression analysis showed that categorized GSH levels had correlation with bleeding on probing (OR = 1.121 [95% CI, 1.025-1.225]) and supuration (OR = 0.155 [95% CI, 0.029-0.838]). Therefore, positive correlations among periodontal parameters, MN, NA, and salivary oxidative stress biomarkers (GSH and UA) reflects worse periodontal conditions and of the type 2 diabetes mellitus parameters. Salivary biomarkers (GSH and UA) played an important role in the detection the functioning of the redox balance and nuclear damage in cheek epithelial cells.

Keywords: oxidative stress, DNA damage, periodontitis, type 2 diabetes mellitus.

LISTA DE SIGLAS E ABREVIATURAS

AGEs – Produtos finais de glicação avançada

CA – Índice de cálculo dentário

CAT – Catalase

DM – Diabetes mellitus

DM – Diabetes mellitus tipo 1

DM2 – Diabetes mellitus tipo 2

DNA – *Deoxyribonucleic Acid*

DP – Doença Periodontal

ECM – Matriz extracelular

EDTA – *Ethylenediamine Tetraacetic acid*

FG – Fluido gengival

GHS – Glutationa reduzida

GPx – Glutationa peroxidase

GRd – Glutationa redutase

GSSG – Glutationa oxidada

HbA1c – hemoglobina glicada

HDL – Colesterol de lipoproteína de alta densidade

IB – Índice de biofilme

IMC – Índice de massa corporal

IL-6 – Interleucina-6

ISG – Índice de sangramento gengival

LPS – Lipopolissacarídeo

MCP-1 – Peptídeo quimioatraente de monócitos 1

MDA – Malondialdeído

MNs – Micronúcleos

NA – Anormalidades nucleares

NCI – Nível clínico de inserção

NFκβ – Fator nuclear de transcrição kappa B

NO – Óxido nítrico

OMS – Organização Mundial da Saúde

OS – Estresse oxidativo

PE – Periodontite

PS – Profundidade de sondagem

PSD – *Polymicrobial Synergy and Dysbiosis*

RAGE – Receptor de produtos finais de glicação avançada

RNS – Espécies reativas de nitrogênio

ROS – Espécies reativas de oxigênio

SOD – Superóxido dismutase

SP – Saúde Periodontal

SS – Sangramento à sondagem

SUP – Índice de supuração

SPSS – *Statistical Package for the Social Sciences*

TAC – Capacidade antioxidante total

TBARs – Substâncias reativas ao ácido tiobarbitúrico

TOTG – Teste oral de tolerância à glicose

TPNC – Terapia periodontal não-cirúrgica

UA – Ácido úrico

8-OHdG – 8-hidroxideoxiguanosina

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1. INTRODUÇÃO

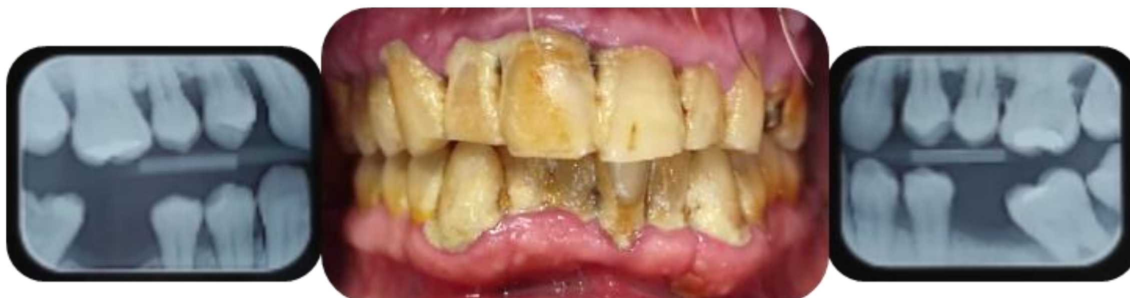
1.1 Doença Periodontal – considerações importantes

A Doença Periodontal (DP) é definida pela sua natureza infecciosa polimicrobiana multifatorial (PAGE *et al.,* 1997) e compreende um grupo de doenças inflamatórias, tais como gengivites e periodontites, que afetam os tecidos de proteção e sustentação dos dentes de indivíduos susceptíveis, respectivamente (AMERICAN ACADEMY OF PERIODONTOLOGY, 1999). Estudos epidemiológicos têm observado que a DP é uma das doenças inflamatórias crônicas humanas mais frequentes na população mundial. Estima-se que 30 a 50% dos adultos com mais de 30 anos sejam acometidos por esta doença (SHEIHAM & NETUVELI, 2002; DYE, 2012) e é mais prevalente em homens e em pessoas de etnia africana (PIHLSTROM *et al.*, 2005). Além disto, corresponde à segunda maior causa de perda dentária no mundo (PETERSEN e OGAWA, 2005), inclusive no Brasil, perdendo apenas para a cárie dentária (MS, 2011); é a segunda doença bucal de maior prevalência em humanos, já que 70% da população mundial apresenta um ou mais danos nos tecidos periodontais (OPPERMANN *et al.*, 2015).

A DP tem início quando a hemostasia entre a microbiota oral e o hospedeiro é desregulada e, atualmente, novos modelos de etiopatogênese têm sido propostos. No modelo conhecido como Sinergismo e Disbiose Polimicrobiana ou *Polymicrobial Synergy and Dysbiosis* (PSD), ocorre o estabelecimento de uma microbiota clímax que abre portas para o crescimento desordenado de algumas bactérias patogênicas, conhecidas como patobiontes, provocando uma quebra do equilíbrio no microambiente periodontal devido à produção de fatores de virulência, que induzem inflamação e destruição dos tecidos periodontais. Com um novo ambiente patogênico e a susceptibilidade do hospedeiro causada por uma alteração da resposta imunológica, a disbiose microbiana se estabelece (PAGE *et al.,*1997; LAMONT e HAJISHENGALLIS, 2015; KINANE *et al.*, 2017).

A periodontite, que é a DP destrutiva, é iniciada pela colonização bacteriana, seguida pela proliferação e extensão do biofilme junto ao ambiente subgengival (KINANE, 2001). Simultaneamente a essa progressão apical, ocorre uma destruição tecidual que envolve metabólitos bacterianos, como enzimas e endotoxinas que, juntamente com a resposta tecidual do hospedeiro, compõem o processo inflamatório da

doença (RYLEV e KILIAN, 2008). Essa alteração se caracteriza pela perda de inserção do ligamento periodontal, destruição do osso alveolar adjacente, com formação de bolsas periodontais, podendo resultar em mobilidade excessiva e levar à perda dental (LÖE, 1993; SOCRANSKY *et al.*,1998; LINDHE *et al.*, 2018).



Fonte: Próprio autor

Figura 1. Imagens de pacientes com diagnóstico de periodontite. Na foto intraoral, observa-se elevada quantidade de cálculo e biofilme supragengivais na maioria dos dentes, bem como edema e inflamação gengival. As radiografias interproximais evidenciam a perda óssea alveolar generalizada e presença de cálculo subgengival.

Portanto, a periodontite é uma doença resultante de uma disbiose significativa da microbiota subgengival, que ocasiona um quadro inflamatório crônico com destruição do tecido periodontal de suporte, no qual há um acúmulo de biofilme nos tecidos mais profundos, causando uma perda de inserção por destruição do tecido conjuntivo e por reabsorção do osso (LAMONT e HAJISHENGALLIS, 2015; BARTOLD e VAN DYKE, 2013; KINANE *et al.*, 2017).

Essa doença tem um curso de progressão lenta a moderada, porém, episódios de destruição mais rápidos podem ocorrer em modificação por doenças sistêmicas e fatores ambientais, ocorrendo com maior frequência em adultos, entre 30 e 65 anos (MACHTEI *et al.*, 1992). Sua manifestação e progressão são influenciadas por uma grande variedade de determinantes, que incluem fatores sociais, comportamentais, sistêmicos, genéticos, dentre outros, constituindo o caráter multifatorial da periodontite. Suas manifestações clínicas são dependentes das propriedades agressoras dos micro-organismos e da capacidade do hospedeiro em resistir à agressão (PAGE e KORNMAN, 1997; OPPERMANN *et al.*, 2012). O fumo, por exemplo, é categorizado como o mais

importante fator de risco comportamental para o desenvolvimento e progressão da DP (AKL *et al.,* 2007; CATON *et al.*, 2018; NASCIEMNTO *et al.*, 2018).

As DPs são de difícil classificação, especialmente a periodontite, devido à grande variabilidade na apresentação clínica, assim como em sua extensão e gravidade em todas as faixas etárias. Com tudo isso, o diagnóstico da doença ainda permanece sendo clínico (PAPAPANOU *et al.,* 2018). Segundo a classificação vigente da doença periodontal, proposta no *2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions*, as periodontites podem ter diferentes níveis de gravidade e extensão. Em relação à gravidade e complexidade de manejo do paciente, podem ser categorizadas em Estágio I (periodontite inicial), Estágio II (periodontite moderada), Estágio III (periodontite avançada com potencial para perda dentária) e Estágio IV (periodontite avançada com potencial para a perda da dentição). Em relação à extensão podem ser localizadas (quando envolvem menos de 30% dos sítios periodontais), generalizadas (> 30% dos sítios), e padrão incisivo-molar. Em relação à progressão e resposta terapêutica, podem ser graduadas em grau A (perda de progressão lenta), grau B (perda de progressão moderada) e grau C (perda de progressão avançada) (CATON *et al.,* 2018).

De acordo ainda com essa classificação, o grau de progressão e resposta terapêutica podem ser modificados por fatores de risco, como: fumo, sendo que pacientes não fumantes correspondem ao grau A, os que fumam 10 cigarros ou menos ao dia grau B e os que fumam mais de 10 cigarros por dia ao grau C; em relação ao diabetes, grau A corresponde aos que não possuem essa condição metabólica, grau B aos que apresentam hemoglobina glicada (HbA1c) < 7% e grau C, HbA1c ≥ 7% (CATON *et al.,* 2018).

Ao longo dos anos, inúmeras evidências ligam a periodontite a muitas doenças sistêmicas, incluindo DM2, doenças cardiovasculares, infecção do trato respiratório, doença renal crônica, resultados adversos da gravidez, doenças neurodegenerativas e câncer. Portanto, a discussão da interrelação entre periodontite e a resposta sistêmica é um ponto importante a ser discutido dentro e fora do campo da Odontologia (BUI *et al.*, 2019).

1.2 Periodontite e alterações sistêmicas

O conhecimento mais apurado sobre a etiologia e a patogênese da doença periodontal e sua natureza crônica, inflamatória e infecciosa leva ao reconhecimento de seus potenciais efeitos deletérios sobre outros órgãos e tecidos (JAIN *et al.*, 2021).

Dados do final da década de 80 levaram à criação da expressão Medicina Periodontal pela primeira vez, quando um grupo de cientistas publicou observações pertinentes sobre a possível associação de periodontite com prematuridade e bebês com baixo peso ao nascimento quando gestantes apresentavam periodontite durante o período gestacional (BECK *et al.*, 2019). Diversos estudos sugeriram a teoria da infecção focal na qual um micro-organismo pode migrar para partes adjacentes ou distantes do corpo. Nos estágios avançados da doença, as bactérias patogênicas do biofilme dentário e seus produtos metabólicos podem entrar na circulação sistêmica durante a mastigação ou procedimentos mecânicos. Além disso, o conceito de medicina periodontal como uma nova disciplina que se concentra em validar esta associação e seu mecanismo biológico através de animais e humanos também foi colocada em pauta (FALCAO e BULLÓN, 2019).

A relação entre a doença periodontal e as doenças sistêmicas vem sendo discutida por mais de 20 anos de estudos e mostra o papel etiológico e modulador da DP nas doenças cardiovasculares (WILLIAMS e OFFENBACHER, 2000; BECK *et al.*, 2019), no diabetes mellitus (NELSON *et al.*, 1990; OLIVEIRA e BARBOSA, 2020; OLIVEIRA *et al.*, 2020), em resultados adversos na gravidez, artrite reumatóide (WILLIAMS e OFFENBACHER, 2000; BECK *et al.*, 2019), doença renal crônica (VILELA *et al.*, 2011; CRAIG *et al.*, 2016), hipoalbuminemia (AMITHA *et al.*, 2012; PATIL *et al.*, 2015), anemia (CARVALHO *et al.,* 2014) e doenças neurodegenerativas, como Alzheimer (DOMINY *et al.*, 2019).

A destruição dos tecidos periodontais parece resultar de uma interação complexa entre periodontopatógenos e o sistema imunológico dos hospedeiros (RYLEV e KILIAN, 2008). O epitélio ulcerado atua como uma porta de entrada para as bactérias acessarem o tecido conjuntivo e chegar à circulação sistêmica. A bacteremia tem sido observada em pacientes com periodontite, tornando-se diretamente relacionada à gravidade da inflamação (LOWE, 2001).

A periodontite não é mais considerada uma doença isolada, pois transcende seu efeito além do local de sua infecção na cavidade oral (JAIN *et al.*, 2021). Em estágios avançados da periodontite, o estado de inflamação crônica pode surgir assim que as células imunes se tornam incapazes de controlar a propagação de bactérias patogênicas. Posteriormente, essa inflamação crônica determina a ligação bidirecional entre periodontite e complicações sistêmicas (CECORO *et al.*, 2020; HAJISHENGALLIS e CHAVAKIS, 2021).

As citocinas e quimiocinas presentes no desafio microbiano conduzem a migração de leucócitos para os tecidos periodontais onde essas células desempenham um papel importante na destruição de agentes patogênicos através da liberação de mediadores de resposta inflamatória local. Esses e outros mediadores são detectados em níveis elevados na saliva, sendo assim considerada um fluido com biomarcadores aceitáveis para determinar alguns aspectos da DP (KEJRIWAL *et al.*, 2014).

A patogênese da DP consiste em uma cascata de reações inflamatórias e imunológicas, que ainda não foram completamente elucidadas (CORREA *et al.,* 2010). Aspectos protetores da resposta do hospedeiro em relação à inflamação incluem recrutamento de macrófagos, células natural killer, células dendríticas, neutrófilos polimorfonucleares (PMNs, do inglês *"polymorphonuclear neutrophils"*) e outras células fagocitárias e, possivelmente, a liberação de citocinas anti-inflamatórias, incluindo o fator de crescimento transformador beta (TGFB), interleucina 4 (IL-4) e interleucina 10 (IL-10) para os tecidos infecto-afetados (YAZDI *et al.*, 2013). Em circunstâncias normais, essas células fagocitárias englobam o patógeno e neutralizam a infecção, resultando mais tarde em apoptose do micro-organismo; além disso, a remoção de células apoptóticas sinaliza uma mudança de fenótipos pró para anti-inflamatórios, reduzindo assim a inflamação e restaurando a integridade dos tecidos (FOX *et al.*, 2010).

Em alguns casos, mesmo após a remoção do patógeno, a cascata de inflamação não cessa e isso leva a uma inflamação descontrolada e crônica pela hiperativação dos leucócitos residentes e células endoteliais, devido ao desafio microbiano persistente (LAMONT e HAJISHENGALLIS, 2015; JAIN *et al.*, 2021). Quando há lesão estabelecida ocorre a transição da resposta imunológica de inata para adquirida, com predomínio de macrófagos, células plasmáticas e linfócitos T e B (CEKICI *et al.*, 2014; HAJISHENGALLIS, 2015). Durante a progressão da doença, o epitélio gengival perde

sua integridade e a sua função de barreira física inata contra disseminação sistêmica de bactérias orais. Devido a isso, os tecidos e capilares sanguíneos entram em contato direto com os patógenos periodontais e suas enzimas metabólicas que passam a ter acesso à circulação sanguínea e produzem mediadores inflamatórios responsáveis pela inflamação sistêmica (VAN DYKE *et al.*, 2021).

Compreende-se que o mecanismo pelo qual as afecções periodontais influenciam a manifestação sistêmica pode ser dividido em 3 partes: migração oral-hematogênica de patógenos periodontais e seus efeitos diretos aos órgãos-alvo (TONETTI e VAN DYKE, 2013); migração transtraqueal de patógenos periodontais e seus efeitos diretos em órgãos-alvo; migração oral-hematogênica de mediadores inflamatórios, como citocinas e anticorpos com seus efeitos em órgãos distantes (IGARI *et al.*, 2014). Essa evidência suporta que o mecanismo representa evidências substanciais de que a periodontite pode afetar o controle glicêmico e expressar o caminho da verdadeira comorbidades causal entre periodontite e DM mal controlada (POLAK *et al.*, 2020).

1.3 Diabetes mellitus – Definição e caracterização

Diabetes Mellitus (DM) é um grupo heterogêneo de distúrbios metabólicos e é caracterizado por insuficiência relativa ou absoluta de secreção de insulina e/ou resistência concomitante à ação metabólica da insulina nos tecidos-alvo, resultando em altos níveis de glicose no sangue (MANFREDI *et al.*, 2004).

O DM pode apresentar sintomas característicos como sede, poliúria, visão turva e perda de peso, além disso, infecções fúngicas genitais ocorrem com frequência. As manifestações clínicas mais graves são cetoacidose ou um estado hiperosmolar não cetótico que pode levar à desidratação, coma e, na ausência de tratamento eficaz, morte (ZIMET *et al.,* 2001).

O DM tipo 1 (DM1) resulta de uma deficiência absoluta de insulina, que é mais comumente causada pela destruição auto imunológica das células beta pancreáticas produtoras de insulina. Já no DM tipo 2 (DM2), músculo, gordura e outras células tornam-se resistentes às ações da insulina. Isso resulta na ativação de um mecanismo compensatório que induz as células beta do pâncreas a secretar mais insulina, porém esse mecanismo deve ser suficiente para manter os níveis de glicose no sangue dentro de um intervalo fisiológico normal (WU *et al.,* 2015). Para que os mecanismos moleculares

envolvidos na síntese e liberação de insulina nos tecidos funcionem normalmente é necessário que a ação e liberação de insulina esteja suprindo à demanda metabólica. Não havendo o correto funcionamento desses mecanismos, pode ocorrer a falência das células beta do pâncreas, seguida da redução da secreção de insulina que leva à hiperglicemia do DM2 (GALICIA-GARCIA *et al.,* 2020).

Porém, a etiologia e a patogênese do DM podem estar envolvidas com defeitos específicos subjacentes mais complexos e que ainda não estão bem compreendidos. Alguns deles já foram identificados, como, por exemplo, anormalidades genéticas que resultam em problemas na secreção de insulina; outros permanecem desafiadores, pois estão cada vez mais envolvidos com uma complexa interação de fatores genéticos, epigenéticos, processos proteômicos e metabolômicos. Assim, a identificação dessas anormalidades é um avanço na compreensão da etiopatogênese do DM (OMS, 2019).

A Organização Mundial da Saúde (OMS) definiu e organizou os grupos com alto risco para DM, a fim de diminuir a morbidade e reduzir a influência da doença e para se tornar de conhecimento dos profissionais que manejam esses pacientes, da seguinte forma: idade ≥ 45 anos e sedentarismo, índice de massa corporal (IMC) ≥ 24 kg/m^2, tolerância à glicose diminuída ou glicose em jejum alterada, histórico familiar com a doença, colesterol de lipoproteína de alta densidade (HDL) mais baixo ou hipertrigliceridemia (HTG), hipertensão ou doenças cardiovascular e cerobrovascular, gestação com mais de 30 anos (OMS, 2019).

Em relação ao diagnóstico para DM, quatro testes atualmente são recomendados: medição de glicose no plasma em jejum, glicose plasmática pós-carga de 2 horas (2 h) após um teste oral de tolerância à glicose (TOTG) com 75 g de glicose oral, hemoglobina glicada (HbA1c) e uma glicemia aleatória na presença de sinais e sintomas de DM. Indivíduos são considerados portadores de DM quando, em jejum, o valor de glicose plasmática é ≥7,0 mmol/L (126 mg/dL), 2 H pós-carga de glicose plasmática ≥11,1 mmol/L (200 mg/dL), HbA1c ≥6,5% (48 mmol/mol) ou uma glicemia aleatória ≥11,1 mmol/L (200 mg/dL) na presença de sinais e sintomas. Se valores elevados forem detectados em pessoas assintomáticas, recomenda-se repetir o teste assim que possível em um dia subsequente para confirmar o diagnóstico (WHO, 2011).

1.3.1 Diabetes mellitus tipo 2 (DM2)

DM2 é um problema de saúde global comum e grave que evoluiu em associação com o crescimento tecnológico e com as rápidas mudanças culturais, econômicas e sociais, populações envelhecidas, crescente urbanização não planejada, mudanças na dieta, como aumento do consumo de alimentos altamente processados e bebidas adoçadas com açúcar, obesidade, atividade física reduzida, estilo de vida e padrões comportamentais pouco saudáveis, desnutrição fetal e aumento da exposição fetal à hiperglicemia durante a gravidez (WHO, 2016).

Esse tipo de DM é o mais frequente na população e acomete 90% dos indivíduos portadores da doença, sendo mais comum em pessoas a partir de 40 anos com sobrepeso, sedentarismo, tabagismo, episódios depressivos e devido a fatores genéticos, embora também tenha crescido o número de crianças e adolescentes acometidos pela doença (ADA, 2018; OLIVEIRA *et al.,* 2020). Acredita-se que estilos de vida sedentários, dietas altamente calóricas, envelhecimento populacional, bem como, o aumento global da obesidade sejam os responsáveis por impulsionar a epidemia de DM2 e quadruplicar a incidência e prevalência da doença (CHATTERJEE *et al.,* 2017).

No DM2, os sintomas geralmente não são graves ou podem estar ausentes, devido ao ritmo lento em que a hiperglicemia piora. Como resultado, na ausência de testes bioquímicos, a hiperglicemia suficiente para causar alterações patológicas e funcionais pode estar presente por muito tempo antes de um diagnóstico ser obtido, resultando na presença de complicações no diagnóstico e maior risco de desenvolver complicações macro e microvasculares (YOON *et al.,* 2006). Estima-se que uma porcentagem significativa dos casos de DM (30 a 80%, dependendo do país) não são diagnosticados (ZIMET *et al.*, 2001).

Indivíduos com DM2 mostram-se, em grande parte, obesos e com elevado percentual de gordura corporal na região abdominal, sendo o tecido adiposo visceral responsável pela resistência insulínica (RI) por meio de vários mecanismos de inflamação, como a liberação de ácidos graxos livres (AGL) e desregulação de adipocinas (CHATTERJEE *et al.,* 2017).

Para a maioria dos indivíduos com esse tipo de DM, o tratamento com insulina não é necessário para a sobrevivência, por isso também ser chamada de diabetes não-insulino dependente, mas para reduzir a glicemia e evitar complicações crônicas pode ser

utilizado (YOON *et al.,* 2006). Estudos destacam a insuficiência vascular periférica como uma complicação crônica da macro e microcirculação associada ao DM2 e que comumente provoca distúrbios de cicatrização e alterações fisiológicas, diminuindo a capacidade imunológica do indivíduo e o tornando mais susceptível às infecções (COSTA *et al*., 2017).

A hiperglicemia constitui o segundo principal fator que contribui inicialmente para a patogênese da aterosclerose e, portanto, para as complicações macrovasculares do DM2. Mais especificamente, aumenta a produção de espécies reativas de oxigênio (ROS), que, por sua vez, inativam o óxido nítrico (NO), contribuindo para a disfunção endotelial e alterações ateroscleróticas (PANENI *et al.,* 2013). Paralelamente a esse mecanismo, as ROS resultam na ativação de outra enzima, a proteína quinase C (PKC). Esta enzima participa na preservação da homeostase vascular através de diferentes mecanismos, incluindo o crescimento celular vascular e apoptose, e a produção de diferentes mediadores, como as citocinas. O resultado é uma intensificação do processo trombótico e, posteriormente, complicações macrovasculares. A PKC, então, induz a produção de ROS e esse ciclo vicioso continua (GERALDES e KING, 2010).

O estado de hiperglicemia crônica contribui para as complicações do diabetes por aumentar o fluxo de frutose-6-fosfato pela via das hexosaminas. A hiperfunção desta via promove a conversão da frutose-6-fosfato em glucosamina-6-fosfato e, como produto final, a uridina difosfato-N-acetil glucosamina (UDP-GIcNAc), que sofre O-glicolisação para o N-acetil-glucosamina (O-GlcNAc) através da enzima O-GlcNAc transferase. O excesso da O-GlcNAc é responsável pelo estímulo e modificação de proteínas celulares e a alteração na expressão gênica, o que resulta no aumento da transcrição de TNF-α e TGF-β e induz a danos em órgãos, como os rins, via OS e superprodução de proteínas de matriz extracelular (ECM, do inglês *"extracellular matrix"*) (REIS *et al.*, 2008; AGHADAVOD *et al.*, 2016).

Outras vias bioquímicas, que estão conectadas com hiperglicemia crônica, incluem a formação de produtos finais de glicação avançada (AGEs) e a expressão do receptor dos AGEs e seus ligantes (BECKMAN *et al.,* 2002). O potencial danoso dos AGEs resulta de alterações diretas nas estruturas e funções das proteínas, já que são frequentemente encontrados na ECM e, portanto, as proteínas da matriz modificada prejudicam as interações matriz-matriz, bem como as interações célula-matriz. Isso pode

causar morte celular, diferenciação celular ou redução da adesão e migração celular (NOWOTNY *et al.*, 2015). A formação dos AGEs modifica diretamente as proteínas intracelulares e prejudica suas funções, através da ligação de AGEs ao receptor de produtos finais de glicação avançada (RAGE) ocasionando danos macro e microvasculares associados às comorbidades do DM2 (CHAVAKIS *et al.*, 2003).

1.3.2 Produtos Finais de Glicação Avançada (AGEs)

Os AGEs são classes variadas de compostos formados em diferentes condições hiperglicêmicas. Estão envolvidos na patogênese do DM2 e nas complicações diabéticas por se formarem nessas condições, por apresentarem um potencial prejudicial ao controle metabólico e por se acumularem no interior das células ou nos tecidos sem atingir a circulação (VLASSARA e URIBARRI, 2014).

A descoberta de uma forma alterada de hemoglobina nos glóbulos vermelhos de pacientes com DM, a conhecida HbA1c, marcou a primeira ligação entre proteínas glicadas e diabetes (NOWOTNY *et al.*, 2015). Tornou-se claro que a ligação não enzimática da glicose ocorre predominantemente com a porção N-terminal valina na cadeia β da hemoglobina. Essa reação entre o grupo amino de uma proteína, lipídio ou ácido nucleico e o grupo carbonila de um carboidrato redutor recebe o nome de reação de Maillard (glicação) (MENEZES *et al.*, 2019) e gera as bases de Schiff, uma imina instável com ligação dupla entre átomos de carbono e nitrogênio e deste com grupo arila ou alquila, que se rearranjam aos produtos Amadori (FU *et al.*, 1996).

Esses produtos são relativamente instáveis, de modo que ocorrem reações consecutivas e paralelas, eventualmente levando à formação dos AGEs irreversíveis em todas as fases da glicação e, também, como produtos intermediários ou subprodutos da auto-oxidação da glicose, peroxidação lipídica ou por compostos carbonílicos altamente reativos, os polióis ou poliálcoois (THORNALLEY *et al.*, 1999).

Nessas condições, os AGEs passam a ser uma das principais fontes de ROS e há crescentes evidências de que o estresse oxidativo (OS) também desempenha um papel fundamental em processos patológicos observados em DM2. O OS está associado às complicações do DM e é o responsável pelas duas características do DM2 (disfunção celular e resistência à insulina), que tem início mesmo antes dos níveis de glicose no sangue atingirem o valor definido como pré-diabetes, devido à superprodução de ROS.

Caso essas alterações não sejam controladas, o indivíduo com pré-diabetes tem a progressão para o DM (HENRIKSEN *et al.*, 2011; VLASSARA e URIBARRI, 2014).

Diversos são os efeitos da circulação de AGEs no organismo, incluindo os danos celulares ocasionados pela a indução da produção de ROS, além de poderem se ligar a receptores celulares específicos, modificando a ECM e alterando interações entre compostos endógenos e a membrana celular de forma a inibir as funções dessas substâncias (SEMBA *et al.*, 2010).

O potencial danoso dos AGEs resulta de alterações diretas nas estruturas e funções das proteínas, ou por ação própria ou devido ao efeito de reticulação de alguns AGEs. Isso pode causar morte celular, diferenciação celular ou redução da adesão e migração celular (HOFMANN *et al.*, 1999). Outros alvos de modificação são as proteínas intracelulares que têm as suas funções prejudicadas devido à formação dos AGEs. Além das mudanças diretas nas estruturas e funções das proteínas, ocorrem danos ligados ao RAGE, pois, os receptores ativam NAPDH oxidases e assim aumenta a formação de ROS intracelulares que leva à formação de AGEs e desencadeia todos os mecanismos danosos, ativando o fator nuclear de transcrição kappa B (NFκβ, do inglês *"nuclear factor kappa beta"*) (BIERHAUS *et al.*, 2001).

A ativação do NFκβ aumenta a expressão de citocinas pró-inflamatórias, como, IL-6 e peptídeo quimioatraente de monócitos 1 (MCP-1), assim como o próprio RAGE, intensificando assim a resposta inflamatória. Para proteger os tecidos dos danos mediados por AGEs, as células podem ativar mecanismos compensatórios, como, por exemplo, processamento de outros receptores de AGEs que se ligam a AGEs extracelulares e medeiam sua captação celular ou mesmo tentar eliminá-los (UEDA *et al.*, 1997).

Apenas agregados de proteínas altamente modificadas por AGEs em tecidos e fluidos corporais são capazes de ativar RAGE. Essas proteínas podem ser formadas através do consumo de alimentos processados termicamente (forma exógena). No entanto, a redução da formação dos AGEs induzida pelo controle da hiperglicemia, bem como, a restrição de itens alimentares ricos em AGEs, são exemplos de abordagens terapêuticas contra os eventos patológicos do DM e doenças associadas, ficando esclarecido o uso dos AGEs como biomarcadores/preditores de complicações do DM (NOWOTNY, *et al.*, 2015).

1.4 Estresse Oxidativo (OS)

O oxigênio é um elemento indispensável para a vida, mas, em determinadas situações, apresenta efeitos deletérios sobre o corpo humano. A maioria dos efeitos potencialmente prejudiciais do oxigênio são devido à formação e à atividade de vários compostos químicos, conhecidos como as ROS, que tendem a doar oxigênio a outras substâncias (LOBO et al., 2010).

Em um estado fisiológico, a formação de radicais livres ocorre continuamente nas células, como consequência de reações enzimáticas (cadeia respiratória, na fagocitose, na síntese de prostaglandinas e no sistema do citocromo P-450) e não enzimáticas (oxigênio com compostos orgânicos e aqueles iniciados por reações ionizantes) e os produtos são essenciais para vários processos biológicos, como sinalização de transdução celular e controle da expressão gênica (LOBO et al., 2010). Nesse quadro, há um equilíbrio dinâmico entre a atividade das ROS e a capacidade de defesa antioxidante, cumprindo as funções metabólicas relevantes. No entanto, quando esse equilíbrio muda a favor das ROS, seja por uma redução na defesa antioxidante ou pelo aumento na produção ou na atividade delas, resulta em OS (SIES, 2015).

Dessa forma, o OS é caracterizado por um desequilíbrio persistente nas reações de oxidação-redução celular, que é comumente encontrado em processos etiológicos de numerosas patologias crônicas não-transmissíveis (BARBOSA *et al.*, 2010). Pode ocorrer efeitos deletérios em biomoléculas, que incluem modificações de bases nos ácidos nucleicos, lipídios, oxidação de resíduos de aminoácidos específicos, formação de carbonilas e oxidação de DNA, levando a mudanças no padrão de expressão gênica (VALKO *et al.*, 2007). Além disso, as ROS podem interagir com receptores ou moléculas intracelulares, ativando ou inativando vias de sinalização, que podem alterar eventos celulares como proliferação, apoptose, angiogênese, dentre outros (TURRENS, 2003).

Os alvos das ROS são os carboidratos, lipídios e proteínas constituintes das membranas celulares e das membranas internas das organelas, tais como, mitocôndrias, retículo endoplasmático e núcleo. Para amenizar ou combater os efeitos deletérios ocasionados pela atividade crônica de ROS, o organismo dispõe de eficientes mecanismos de reparo antioxidante que são capazes de impedir ou retardar a ação dessas moléculas sobre as células (HALLIWELL e WHITEMAN, 2004).

O sistema antioxidante celular exerce um papel importante na detoxificação das ROS, proporcionando um equilíbrio entre a produção e a degradação dessas moléculas. Esse sistema é dividido em enzimático, não enzimático e alguns autores defendem o estudo das substâncias com capacidade antioxidante total (TAC, do inglês *"total antioxidante capacity"*) (GHISSELLI *et al.*, 2000).

O sistema antioxidante enzimático é composto, principalmente, em termos quantitativos, pelas seguintes enzimas antioxidantes: superóxido dismutase (SOD), glutationa peroxidase (GPx) e catalase (CAT) (BARBOSA *et al.*, 2010). A SOD é uma metaloenzima que catalisa a dismutação do ânion superóxido em oxigênio molecular e peróxido de hidrogênio (H_2O_2). Há três isoformas de SOD (SOD I, SOD II e SOD III), que são caracterizadas de acordo com os seus grupamentos metálicos: Cobre/Zinco (Cu/Zn-SOD, SOD I), Manganês (Mn-SOD, SOD II) e Ferro (Fe-SOD, SOD III). A SOD I é encontrada, principalmente, no citosol, a SOD II nas mitocôndrias e a SOD III no espaço extracelular. Essa última corresponde à maioria da atividade SOD no plasma, linfa e líquido sinovial. Em geral, a SOD é distribuída em todo o organismo, mas apresenta altas concentrações no fígado, cérebro, rins, coração e eritrócitos. A GPx é uma outra enzima antioxidante, responsável pela conversão de peróxido de hidrogênio (H_2O_2) em água (H_2O) e radical hidroxila (-OH). A CAT, por fim, é uma enzima pertencente à subclasse das oxidorredutases, e seu papel é o de decompor o H_2O_2 em H_2O e O_2 (VASCONCELOS *et al.*, 2007).

Dentre os representantes do sistema antioxidante não enzimático, destaca-se a glutationa reduzida (GSH ou γ-glutamil-L-cisteinilglicina). A GSH é um tripeptídeo, detectado em todos os tecidos, principalmente na sua forma reduzida. Ela é um antioxidante que atua na manutenção do balanço redox da célula e na defesa contra agentes eletrofílicos. Essa capacidade antioxidante se dá pelo grupamento tiol (SH) reativo de sua cisteína. Além disso, a GSH é um substrato da GPx que gera o dissulfeto de glutationa ou glutationa oxidada (GSSG, do inglês *"glutathione disulfide"*) (BEHARD e KRAUSE, 2007). A relação GSSG/GSH é o melhor biomarcador de estresse oxidativo celular, e seu aumento pode refletir maior degradação de GSH em reações redox, à custa de uma síntese menor (ARANA *et al.*, 2017). Para a manutenção do ambiente redutor intracelular, a razão entre glutationa reduzida e oxidada (GSH/GSSG) é mantida em níveis de 100:1. Para evitar a depleção da GSH e aumento da GSSG, a glutationa redutase

(GRd) reduz a GSSG às custas de NADPH, regenerando a GSH e mantendo, desta forma, o estado redox intracelular (BEHARD e KRAUSE, 2007).

O ácido úrico (UA, do inglês "uric acid") é o produto do catabolismo das bases nitrogenadas do grupo das purinas (adenina e guanina) no corpo humano. É predominantemente eliminado pelos rins e excretado pela urina e, quando em elevada quantidade, forma cristais que se depositam nas articulações, líquido sinovial, tendões e tecidos circundantes (XIANG *et al.*, 2014). Na saliva, ele representa em torno de 70-85% da capacidade antioxidante salivar e estudos relatam a sua ação na homeostasia redox, através da eliminação de radicais livres (NAGLER *et al.*, 2002).

Na análise da TAC, leva-se em consideração a ação acumulativa de todos os antioxidantes presentes e se obtém um parâmetro integrado, capaz de revelar aspectos em relação ao delicado equilíbrio redox existente. A capacidade antioxidante total auxilia na avaliação dos fatores nutricionais, fisiológicos e ambientais do balanço redox em seres humanos (GHISSELLI *et al.*, 2000). Outro aspecto é a dualidade das informações obtidas: um aumento da TAC não é, necessariamente, uma condição desejável e nem indesejável, uma vez que pode ocorrer quando há uma diminuição na produção de ROS e espécies reativas de nitrogênio (RNS). O mesmo raciocínio aplica-se a um marcador isolado (PRIOR e CAO, 1999).

Como já mencionado, as ROS ocasionam a peroxidação de ácidos graxos poli-insaturados, formando o radical centrado no carbono (radical PUFA, do inglês *"polyunsaturated fatty acid"*) ou o radical peróxido lipídico que leva à redução de funções da membrana. Outros estudos mostram que substâncias reativas ao ácido tiobarbitúrico (TBARs, do inglês "thiobarbituric acid reactive substances") refletem vários mecanismos diferentes de oxidantes e aponta para um papel de reativos intermediários de oxigênio. A peroxidação lipídica e consequentes produtos de degradação constituintes das TBARs, como malondialdeído (MDA), são vistos em fluidos biológicos e indicam o estado pró-oxidante (OMEH e UZOEGWU, 2010). As ações dos biomarcadores do balanço redox citados anteriormente estão melhor descritas na tabela 1.

As concentrações de proteína total são de vital importância na saliva e desenvolvem o papel de lubrificação, proteção física, limpeza, tamponamento,

manutenção da integridade dos dentes, atribuição do sabor dos alimentos, digestão e atividade antibacteriana (PANCHBHAI *et al.*, 2010). Durante quadros de inflamação no organismo a concentração de proteína total eleva-se em função da ativação do sistema simpático para maior liberação de proteínas com potencial papel protetor da saliva contra as doenças (KEJRIWAL *et al.*, 2014) ou simplesmente pelo vazamento delas durante a passagem de citocinas (HENSKENS *et al.*, 1993).

Existem evidências que ligam a doença periodontal a condições sistêmicas, como o DM, e sabe-se que OS desempenha um importante papel na patogênese de muitas doenças orais e sistêmicas. A relação do OS com a doença periodontal se dá pela produção aumentada de ROS após estimulação por patógenos periodontais e/ou seus componentes, devido à ação não apenas dos neutrófilos, mas também dos monócitos, fibroblastos gengivais e células do ligamento periodontal (GOLZ *et al.*, 2014). Já no DM, é devido ao quadro crônico de inflamação, ocasionado pela hiperglicemia que resulta na maior produção de ROS e desequilíbrio do balanço redox com menor atividade dos sistemas antioxidantes (BONNEFONT-ROUSSELOT, 2002).

Tabela 1. Descrição de alguns biomarcadores do balanço redox

Marcadores	Descrição/Ação	Referências
Antioxidantes não-enzimáticos		
GSH	Atua na biotransformação e eliminação de xenobióticos e na defesa das células contra o OS	COUTO *et al.*, 2016
UA	Capacidade de doar elétrons, combatendo os radicais livres e outras substâncias oxidantes	NAGLER *et al.*, 2002
Antioxidantes Enzimáticos		
SOD	Catalisa a conversão do radical superóxido em peróxido de hidrogênio (H_2O_2)	BEHARD e KRAUSE, 2007
CAT	Catalisa a conversão de H_2O_2 em O_2 e H_2O	VINCENT *et al.*, 2018
GPx	Catalisa a redução do H_2O_2 a H_2O	VASCONCELOS *et al.*, 2007
Capacidade antioxidante		
TAC	Determina o conteúdo de antioxidantes e pode descobrir antioxidantes não identificados ou tecnicamente difíceis de detectar	VINCENT *et al.*, 2018
Derivados da oxidação de lipídeos		
TBARs	Quantificação da formação de MDA	TRIVED *et al.*, 2014
MDA	Aldeído que resulta da oxidação dos ácidos graxos AA, EPA e DHA	OMEH e UZOEGWU, 2010

1.5 Associação entre periodontite e DM2

A periodontite é reconhecida como a sexta maior complicação associada ao DM, (LÖE, 1993). Em pacientes com DM, a periodontite se manifesta com maior extensão e gravidade em comparação a indivíduos sem a alteração metabólica (MEALEY e OATES, 2006).

O efeito das infecções periodontais no DM é explicado pelo aumento de mediadores pró-inflamatórios sistêmicos, que estão envolvidos em mecanismos que resultam na resistência à insulina, exacerbando o quadro hiperglicêmico já existente no portador de DM (LALLA e PAPAPANOU, 2011). O estudo de Taylor *et al*. (1996) relatou que indivíduos com DM2 não-controlada apresentavam maior prevalência de doença periodontal, por serem mais suscetíveis à gengivite e periodontite, e que a gravidade da doença é um fator de risco para obtenção de um controle glicêmico ruim.

Há evidências por estudos longitudinais (DEMMER e PAPAPANOU, 2010; JIMENEZ *et al*., 2012) e por estudos de revisão sistemática e meta-análise (CHÁVARRY *et al*., 2009; NASCIMENTO *et al.*, 2018) que demonstram que a DM é um importante fator modificador da periodontite, avança a progressão da doença em 86% [1,86; 95% IC; 1,3-2,8], levando a perdas mais graves de osso alveolar e aumento do grau de dificuldade para tratá-la, de acordo com a classificação vigente das DP (CATON *et al.,* 2018). O DM2 e as condições relacionadas, como a obesidade, estão associados a alterações de alguns fatores fisiológicos, nutricionais e metabólicos, incluindo hiperglicemia, formação dos AGEs, hiperlipidemia e aumento da adiposidade. Essas alterações têm várias consequências, incluindo a desregulação imunológica, manifestada por um estado inflamatório pronunciado e de longa duração e a autolimitação das células imunológicas (POLAK *et al.*, 2020; RAPONE *et al.*, 2021).

A inflamação crônica de baixo grau é causada tanto pela hiperglicemia, bem como, pela presença de agentes infecciosos no periodonto. Ambos aumentam a concentração sérica de marcadores e células inflamatórias, dentre os quais, a proteína C-reativa (PCR), a interleucina-6 (IL-6) e o fator de necrose tumoral-α (TNF-α) são os responsáveis pelo aumento do nível glicêmico, que é a principal característica da intolerância à glicose (COSTA TEIXEIRA *et al*., 2014). Os lipopolissacarídeos e outras endotoxinas de bactérias aderidas ao periodonto desencadeiam a produção de citocinas

inflamatórias, iniciando a cascata da resposta inflamatória, o que pode agravar o quadro de hiperglicemia, pois aumenta a resistência dos tecidos à insulina e provoca alterações no metabolismo da glicose (COSTA TEIXEIRA *et al.*, 2014).

Por sua vez, o DM influencia o surgimento e progressão da doença periodontal na medida em que o doente descompensado terá dificuldade de cicatrização, alterações vasculares, disfunção dos neutrófilos, diminuição da síntese e renovação de colágeno (BRASIL, 2013; SBD, 2017). Entretanto, apesar do exposto, o fator proeminente pelo qual o DM afeta o periodonto ainda não está totalmente esclarecido. Porém, acredita-se que a relação está baseada no desafio microbiano associado a fatores ambientais, predisposição genética e fatores sistêmicos que podem alterar a resposta imuno-inflamatória do hospedeiro, conforme mostra a Figura 2 (LEITE *et al.*, 2016).

Tem-se demonstrado diretamente a influência da periodontite sobre o DM através de estudos que mostram que o tratamento periodontal não-cirúrgico diminui o volume bacteriano e reduz a inflamação, restaurando a sensibilidade à insulina o que resulta na melhora do controle metabólico (SILVA-BOGHOSSIAN *et al.*, 2014; MAURI-OBRADORS *et al.*, 2015).

Fonte: Fabri *et al.* (2014)

Figura 2. Mecanismo de perda óssea alveolar relacionada ao diabetes na doença periodontal.

Lobão *et al.* (2019) mostraram que o tratamento periodontal não-cirúrgico é capaz de reduzir os níveis de IL-6, porém não foram detectadas grandes mudanças nos níveis de albumina, PCR e leucócitos aos 90 dias após a intervenção periodontal. Demonstrou-se no estudo que a terapia periodontal não-cirúrgica melhora a condição periodontal e reduz os níveis séricos de IL-6 em adultos sistemicamente saudáveis com periodontite. Além disso, melhora nos parâmetros periodontais podem mostrar um efeito sobre o nível de PCR e a porcentagem de basófilos não tão expressivos, mas existentes.

Um estudo de revisão sistemática e meta-análise revelou a redução significativa da hemoglobina glicada e nível de glicose plasmática em jejum em pacientes com DM2 e DP submetidos à terapia periodontal não-cirúrgica, sugerindo o tratamento da DP como forma de controle glicêmico (TESHOME e YITAYEH, 2017).

Mauri-Obradors *et al.* (2018) mostraram em seu estudo com indivíduos que possuem DM2 e periodontite uma melhora do estado periodontal (redução nos parâmetros indicadores de doença, como índice de sangramento gengival (ISG), índice de biofilme (IB) e profundidade de sondagem) e a melhora significativa no controle metabólico (redução na hemoglobina glicada e na glicemia em jejum) aos 6 meses após o tratamento periodontal não-cirúrgico mesmo quando não há mudanças significativas no estilo de vida nem no tratamento médico. Além disso, o estado periodontal e o controle metabólico no grupo controle, que recebeu somente raspagem supragengival, permaneceram inalterados.

Ainda sobre o efeito do tratamento periodontal não-cirúrgico sobre biomarcadores inflamatórios sistêmicos, uma revisão sistemática e meta-análise incluiu 3 estudos com indivíduos com DM2 e periodontite que analisaram parâmetros do OS, porém não houve diferença estatisticamente significante dos níveis dos biomarcadores do estresse oxidativo estudados entre os grupos controle e teste no baseline e nem após o TPNC (SILVA *et al.*, 2018). Koromantizos *et al.* (2012) analisaram d-8-iso em soro desses pacientes e concluíram que o TPNC melhorou os níveis de HbA1c, mas não do marcador de OS que foi estudado. Muthuraj *et al.* (2017) avaliaram 8-hidroxideoxiguanosina (8-OHdG) em fluido gengival (FG) e observaram redução estatisticamente significante do biomarcador avaliado para estudo do OS e dos níveis de HbA1c, com redução máxima 3 meses após a intervenção periodontal. Mizuno *et al.* (2017) observaram melhora no balanço de OS em soro (índice-oxidativo), mas não nos níveis de HbA1c aos 3 meses após a TPNC. Mesmo

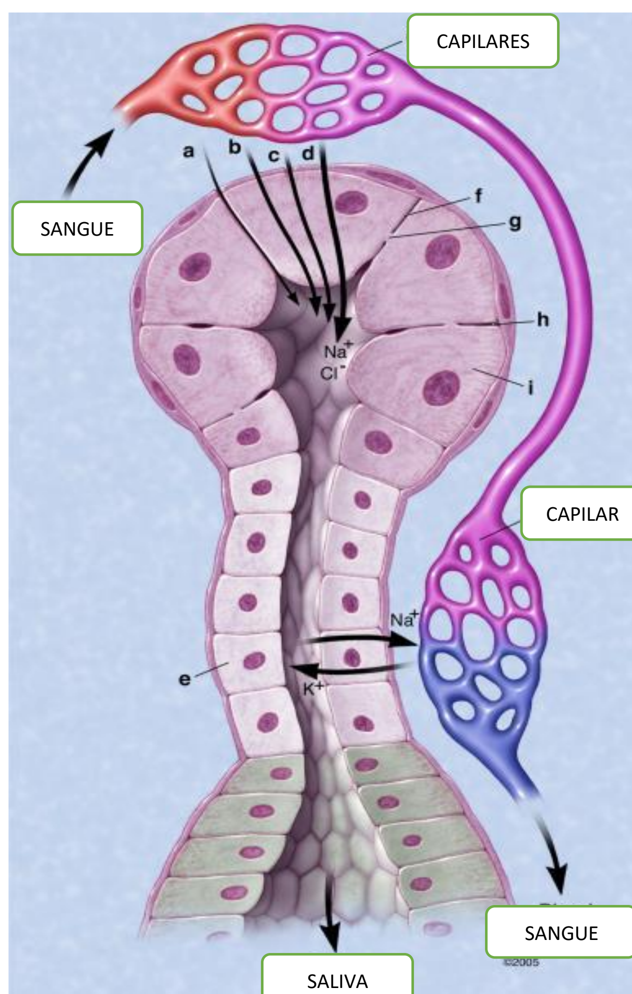
com esses resultados é possível constatar que existe relação entre periodontite e DM2 devido ao aumento de OS.

1.6 Periodontite, DM2 e OS

A saliva humana é um biofluido claro heterogêneo, ligeiramente ácido (pH 6,0 a 7,0), composto de água (99%), proteínas (0,3%) e substâncias inorgânicas (0,2%) (HUMPHREY e WILLIAMSON, 2001). Assim como a saliva, o sangue é um fluido corporal complexo conhecido por conter uma ampla gama de componentes moleculares, incluindo enzimas, hormônios, anticorpos e fatores de crescimento (HOLSINGER e BIU, 2007).

A saliva é gerada dentro das glândulas salivares por células acinares, coletadas em pequenos dutos e posteriormente liberadas na cavidade oral. Cada glândula salivar é altamente permeável e envolta por capilares, que permitem a livre troca de moléculas sanguíneas nas células acinares adjacentes produtoras de saliva (TIWARI, 2011), conforme figura 3.

As glândulas salivares são altamente vascularizadas, permitindo a troca de constituintes do sangue o que possibilita alterações na composição molecular do sangue modificarem a composição das secreções salivares. Os biomarcadores sanguíneos específicos do DM podem alterar suficientemente a produção das glândulas salivares, produzindo biomarcadores da saliva baseados nos distúrbios sistêmicos (YOSHIZAWA *et al.*, 2013), o que faz com que ela possa ser entendida como um importante fluido corporal alternativo para avaliação dos parâmetros do balanço redox (LATHA *et al.*, 2018).



Fonte: Forde *et al.* (2006)

Figura 3. Mecanismo de transporte molecular do soro para os ductos das glândulas salivares.

Na maioria dos casos, o dano relacionado a ROS é controlado, modulado ou removido por processos antioxidantes enzimáticos ou não enzimáticos (SILVA *et al.*, 2018). Os mecanismos antioxidantes enzimáticos regulam diretamente a neutralização dessas ROS (LOBO *et al.*, 2010). Em contraste, os mecanismos antioxidantes não enzimáticos são responsáveis pela redução da concentração de ROS e são representados por vitaminas, bioflavonoides e oligoelementos, obtidos por uma dieta balanceada composta de frutas e vegetais (TAN *et al.*, 2018).

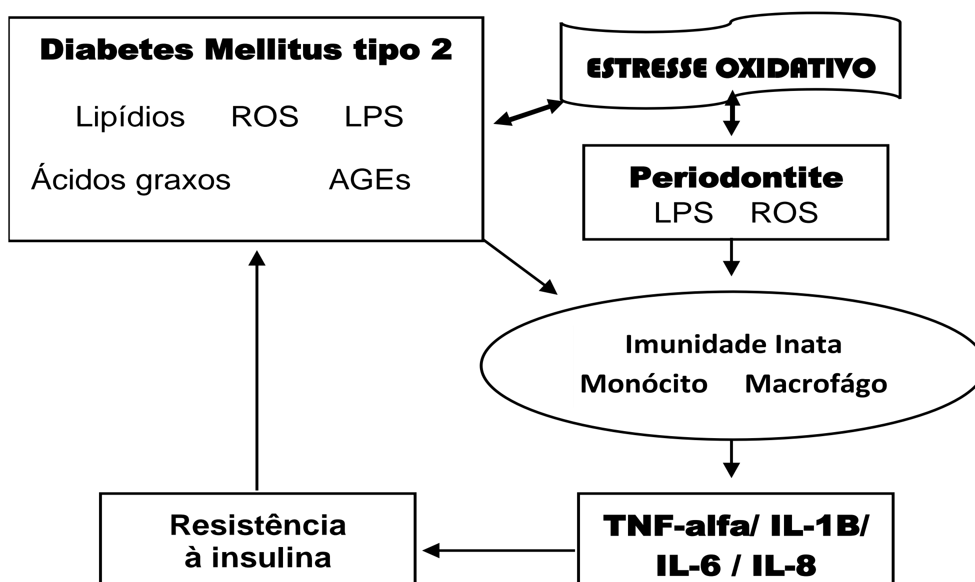
O efeito patogênico da hiperglicemia, possivelmente em conjunto com os ácidos graxos, é mediado através do aumento da produção de ROS e RNS e, subsequente, OS.

Eles oxidam e danificam diretamente o DNA, proteínas, lipídios e carboidratos e prejudicam o sistema endógeno de defesa antioxidante. Além de sua capacidade em danificar diretamente as macromoléculas, estes radicais livres, indiretamente, induzem danos aos tecidos, ativando várias vias celulares sensíveis ao estresse (TURRENS, 2003; GUMUS *et al.*, 2016).

Como descrito anteriormente, a periodontite e o DM2 são doenças com características de inflamação crônica e com os níveis de OS permanentemente aumentado (PRESHAW *et al.*, 2012; SILVA *et al.*, 2015). O aumento das ROS de forma local e sistêmica encontrado nessas patologias, pode afetar significativamente o padrão de funcionamento do sistema imunológico e de diversos tecidos, estando intimamente relacionado às comorbidades associadas ao DM2 (TURRENS, 2003; GUMUS *et al.*, 2016) (Figura 4).

Níveis excessivos de glicose induzem a produção de radicais livres e aumentam o OS pelo aumento da formação dos AGEs. Esses mecanismos patológicos no DM2 com doença periodontal preexistente podem ser responsáveis pela destruição periodontal exacerbada observada em portadores de DM (VINCENT *et al.*, 2018). Os AGEs estão significativamente relacionados à deterioração periodontal associada ao estado metabólico do DM2, mostrando que a análise de seus níveis pode ser um biomarcador adequado para refletir o estado periodontal nesses pacientes (TAKETA *et al.*, 2006).

Nesse contexto, o estudo dos níveis de biomarcadores salivares do OS presentes na periodontite e em DM2 é de fundamental importância para o melhor entendimento dos mecanismos relacionados ao diagnóstico preventivo e controle dessas patologias.



Fonte: Adaptado de Tunes *et al.* (2010)

Figura 4. Influência da atividade das ROS sobre DM2 e periodontite.

1.7 Micronúcleos e anormalidades nucleares

A capacidade regenerativa dos tecidos é fundamental para um envelhecimento saudável. A regeneração depende do número e da taxa de divisão das células em proliferação, chamadas de células basais, de sua estabilidade genômica e de sua propensão para a morte celular (THOMAS *et al.*, 2009).

Níveis elevados de radicais livres e declínio simultâneo em mecanismos de defesa antioxidantes podem levar a danos em enzimas e organelas celulares. Quando há interação bactéria-hospedeiro, como no caso da periodontite, devido à reação bactericida durante a fagocitose dos patógenos, ocorre o aumento do OS por causa de uma maior produção de ROS, como ânion superóxido, radical hidroxila, óxido nitroso e peróxidos de hidrogênio. Isto estimula a produção elevada de radicais superóxido por meio de leucócitos polimorfonucleares, o que resulta na destruição de tecidos periodontais (KIM *et al.*, 2010; SEZER *et al.*, 2012).

As células PMNs produzem e liberam uma grande quantidade de ROS, culminando no aumento do dano oxidativo aos tecidos periodontais como reação aos antígenos bacterianos (SCULLEY e LANGLEY-EVANS, 2002). As ROS são ativas na

despolimerização dos componentes da matriz extracelular, na peroxidação lipídica, na oxidação de enzimas como anti-proteases, na indução de citocinas pró-inflamatórias e em danos ao DNA (CANAKÇI *et al.*, 2006). Essa superprodução de ROS associada à patogênese de alguns distúrbios, como DM2 podem contribuir para a lesão do tecido hospedeiro (SEN, 1995), ocasionando o comprometimento significativo da integridade celular e danos oxidativos às moléculas de carboidratos, lipídios e DNA (CORBI *et al.*, 2014). Assim, em estados hiperglicêmicos, ocorre superprodução de ROS, que, além dos efeitos deletérios já descrito sobre a resposta inflamatória, favorecem a persistência de danos genéticos (BINICI *et al.*, 2013).

O dano ao DNA ocasionado pelos ROS abrange a oxidação de nucleosídeos, o que pode causar quebras na cadeia de DNA. Este tipo de dano pode ter consequências teratogênicas ou carcinogênicas (YFJORD e BODVARSDOTTIR, 2005). Dentre os métodos utilizados para determinar o dano ao DNA, destacamos o ensaio de citoma de micronúcleos (MNs) (THOMAS *et al.*, 2009).

Os MNs são originários de fragmentos de cromossomo ou de cromossomos inteiros que sofreram mutação durante a divisão nuclear. Sua avaliação serve para detecção de eventos clastogênicos (quebra de cromossomos) e aneugênicos (aneuploidia ou segregação cromossômica anormal) e a sua presença indica o efeito mutagênico, evidenciando as anormalidades nucleares (NAs, do inglês *"nuclear abnormalities"*) ou agentes genotóxicos, como os compostos micronucleogênicos (HEDDLE *et al.*, 1991).

Os MNs e NAs podem ser facilmente avaliados em células epiteliais esfoliadas, como células da mucosa oral humana, que é um tecido de fácil acesso para coleta de células de forma minimamente invasiva e não causa estresse indevido aos sujeitos do estudo (THOMAS *et al.*, 2009). Esta análise detecta as aberrações cromossômicas que se proliferam na camada basal do epitélio e depois migram para a superfície epitelial, servindo como um biomarcador de genotoxicidade (HOLLAND *et al.*, 2008).

A detecção de MNs em citologia esfoliativa deve ser interpretada como resultante da exposição recente a carcinógenos ou o reparo frente a erros espontâneos durante a duplicação do DNA. Além dos MNs e botões nucleares (MN com uma ponte ao núcleo principal), também são detectadas as NAs por defeitos citocinéticos (células binucleadas),

potencial proliferativo (frequência celular basal) e/ou morte celular (cromatina condensada, cariorrexe, picnose e cariólise) (THOMAS e FENECH, 2011).

Com base no exposto, percebe-se que OS está intrinsicamente relacionado à patogênese do DM2 e da periodontite e, quando permanentemente aumentado, leva a piores estágios dessas doenças e causa danos no DNA celular. Portanto, torna-se importante avaliar a ocorrência das alterações nucleares e sua relação com essas doenças, bem como, se há ou não diferenças nos níveis dos biomarcadores do balanço redox entre indivíduos com DM2 com e sem periodontite. Sabendo-se que a saúde bucal é um aspecto inerente ao estado geral de saúde de um indivíduo, estudos que associem a condição bucal e fatores dentários com dano ao DNA celular e biomarcadores sistêmicos são necessários para elucidar essa relação.

2. JUSTIFICATIVA

Diversos estudos têm relatado que, quando ocorre a quebra da homeostase, devido a uma maior concentração de ROS ou uma diminuição na capacidade antioxidante, gera-se o OS. Essa condição está relacionada a diversas alterações sistêmicas que pode estar envolvida na patogênese das doenças periodontais, pois a elevada produção de ROS pelas células inflamatórias pode promover a destruição tecidual. O efeito patogênico de oxidação, devido à hiperglicemia no DM2 danifica diretamente o DNA, proteínas, lipídios e carboidratos, e por isso, prejudica o sistema endógeno de defesa antioxidante.

Periodontite e DM2 são doenças com características de inflamação crônica e OS permanentemente aumentado o que pode afetar significativamente o padrão de funcionamento de diversos tecidos, o que está intimamente relacionado às comorbidades associadas ao DM2 (GUMUS *et al.*, 2016). Também é relatado aumento de dano oxidativo (SILVA *et al.*, 2015) e níveis mais baixos de antioxidantes (BALTACIOĞLU *et al.,* 2014) em pacientes com periodontite em comparação a indivíduos periodontalmente saudáveis. As concentrações locais e sistêmicas de alguns biomarcadores de OS podem explicar a condição inflamatória periodontal (SILVA *et al.,* 2018), porém não se sabe ao certo o quão danoso a presença do DM2 associado ou não à periodontite pode ser ao material genético celular. Faz-se necessário mais estudos com o objetivo de contribuir sobre essa temática, tendo em vista que não existem estudos na literatura que comparem a presença de danos ao DNA em células da mucosa jugal com o comportamento das substâncias oxidantes e agentes antioxidantes em indivíduos com periodontite com ou sem DM2 e periodontalmente e sistemicamente saudáveis.

3. PROPOSIÇÃO

3.1. Objetivo Geral

O presente estudo tem como objetivo avaliar os níveis de alguns dos principais biomarcadores salivares do estresse oxidativo e a presença de anormalidades nucleares em pacientes com periodontite com ou sem DM2 e periodontalmente e sistemicamente saudáveis.

3.2. Objetivos específicos

- Identificar através de uma revisão sistemática se há ou não diferença entre os níveis de estresse oxidativo em indivíduos com DM2 com ou sem periodontite.

- Analisar na saliva os níveis das enzimas antioxidantes glutationa reduzida (GSH), ácido úrico (UA) e capacidade antioxidante total (TAC), da peroxidação lipídica, através das substâncias reativas ao ácido tiobarbitúrico (TBARs) e oxidação de proteínas, pelo estudo das proteínas totais.

- Avaliar o dano ao DNA de células epiteliais da mucosa jugal, utilizando o teste de micronúcleos (MNs) e presença de anormalidades nucleares (NAs);

- Correlacionar os biomarcadores do estresse oxidativo, as anormalidades nucleares, os parâmetros periodontais e os laboratoriais entre os grupos de estudo.

4. METODOLOGIA

4.1 Amostra Populacional

Os participantes incluídos no estudo foram recrutados da Clínica de Periodontia da Faculdade de Odontologia da Universidade Federal do Rio de Janeiro (UFRJ), RJ, Brasil e do setor de Nutrologia do Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro (UFRJ), RJ, Brasil, entre o período de fevereiro de 2020 a maio de 2022.

A amostra foi constituída por indivíduos acima de 18 anos, ambos os gêneros e com 10 ou mais dentes, distribuídos nos grupos: sistemicamente e periodontalmente saudáveis (HC), com periodontite e sem alterações sistêmicas (PE) e com DM2 e periodontite (DPE). Foram considerados pacientes com saúde periodontal os que apresentavam menos de 10% dos sítios com sangramento à sondagem (SS) e profundidade de sondagem (PS) e/ou nível clínico de inserção (NCI) ≤ 3 mm, podendo apresentar PS ou NCI = 4 mm em até 5% dos sítios, sem SS concomitante. Pacientes diagnosticados com gengivite tinham ≥ 10% dos sítios com SS e PS e/ou NCI ≤ 3 mm, podendo apresentar PS ou NCI = 4 mm em até 5% dos sítios, sem SS concomitante. Portadores de periodontite apresentavam no mínimo 10% dos dentes com NCI e/ou PS ≥ 5 mm, ou ≥ 15% dos dentes com NCI e/ou PS ≥ 4mm, e SS concomitante (DA SILVA BOGHOSSIAN *et al.,* 2011; CATON *et al.,* 2018).

Os critérios de exclusão foram: portadores de DM1, de doença renal crônica, de neoplasias malignas, de Síndrome da Imunodeficiência Adquirida, de doenças auto-imunes; serem gestantes ou lactantes; serem imunossuprimidos por medicação; possuírem evidência de outras doenças sistêmicas ASA III e IV; possuírem alterações no fluxo menstrual; serem fumantes ou ex-fumantes há menos de vinte anos; serem usuários de aparelho ortodôntico; ter história de uso de anti-inflamatórios esteroidais ou não esteroidais nos três meses anteriores e durante o estudo; usarem antibióticos ou quaisquer suplementos dietéticos, incluindo suplementos antioxidantes nos últimos seis meses antes do estudo, seguirem qualquer dieta específica além de suas dietas habituais durante os últimos seis meses e terem recebido raspagem supra e subgengival e/ou cirurgia periodontal nos seis meses anteriores ao estudo.

Antes do início da anamnese e coletas, todos os pacientes foram esclarecidos quanto a seus riscos e benefícios, leram e assinaram o Termo de Consentimento Livre e Esclarecido. Este estudo foi aprovado pelo Comitê de Ética do Hospital Universitário Clementino Fraga Filho da UFRJ (n. 3.807.461) (Anexo I).

O cálculo do tamanho amostral foi realizado com os desvios-padrão para capacidade antioxidante total (TAC) apresentados na literatura (PENDYALA *et al.*, 2020). Assim, utilizou-se o desvio-padrão de 0,13 para grupo controle (A) e de 0,09 para grupo teste (B), estimando-se uma diferença de 0,1 µmol/dl na medição da TAC entre os grupos. Esse cálculo foi realizado no WinPepi software (http://www.brixtonhealth.com/pepi4windows.html) com nível de significância de 5% e poder de 80%, que forneceu o seguinte *output*:

REQUIRED SAMPLE: Total 40 (20 in A, 20 in B)

EXPECTED PRECISION:

Approx. 80% CI for difference between means (D) = D - 0.070 to D + 0.070

4.2 Exame Clínico Periodontal

Os exames clínicos foram realizados nas instalações da Disciplina de Periodontia do Departamento de Clínica Odontológica da FO-UFRJ, além de aplicação de anamnese médica e odontológica. As medidas clínicas periodontais foram realizadas com espelho plano número 5 e sonda periodontal milimetrada Carolina do Norte (UNC-15, Hu-Friedy, Chicago, IL, EUA). Todos os exames foram executados por um único examinador treinado e calibrado (autor da tese). Os seguintes parâmetros clínicos periodontais foram avaliados: índice de biofilme (IB), SS, ISG, Índice de Cálculo dentário (CA), Índice de supuração (SUP), além das medidas de PS e o NCI em mm (MACHTEI *et al.*, 1992). Essas medições foram realizadas em 6 sítios por dente (mésio-vestibular, vestibular, disto-vestibular, mésio-lingual, lingual, disto-lingual), de todos os dentes, com exceção dos terceiros molares.

4.3 Reprodutibilidade Intra-Examinador

A calibração do examinador envolveu o exame de 5 indivíduos com diagnóstico de periodontite moderada a avançada, que não participaram do estudo. Os exames periodontais foram realizados em cada indivíduo com o intervalo de 2 horas entre eles. Examinou-se 6 sítios (Disto-Vestibular/Vestibular/Mésio-Vestibular/Disto-Palatino ou Disto-Lingual/Palatino ou Lingual/Mésio-Palatino ou Mésio-Lingual) com a sonda periodontal milimetrada mencionada acima em quadrantes diagonais de cada paciente (quadrantes 1 e 3 ou 2 e 4) (DOWSETT *et al.*, 2002). Foi calculado o coeficiente de correlação intraclasse (ICC) para os parâmetros PS e NCI, com valores de 91,1% e 89%, respectivamente, com uma diferença ≤1 mm.

4.4 Coleta e separação das amostras

As amostras de saliva foram coletadas no período da manhã (10:00H-12:00H). Os indivíduos não comeram e nem beberam, exceto 300 mL de água mineral (não gaseificada) 1 hora antes da coleta por todos os participantes, para excluir qualquer influência da mastigação ou alimentos. A saliva foi transportada ao laboratório de microbiologia oral da UFRJ para serem aliquotadas em Eppendorfs de 2 mL, onde foram centrifugadas a 10.000 g por 10 min a 4° C para remover debris. Os debris foram descartados e cada sobrenadante transferido separadamente para outro eppendorf, sendo armazenados a -80° C até a análise. As análises foram realizadas no laboratório de Fisiopatologia do Exercício da Universidade Estadual do Rio de Janeiro (UERJ), sob comando do Professor Doutor Gustavo Casimiro Lopes.

4.5 Análise das amostras

A glutationa reduzida (GSH) foi inserida em 100 mL de saliva pipetada em microtubos contendo 200 mL de solução de ácido tricloracético (TCA) 10% que foi misturada e centrifugada a 4000 *g* por 10 min a 16 ºC. Para 200 mL do sobrenadante 700 mL de tampão de Tris-HCl 400 mM pH 8,9, foi adicionado, seguido pela adição de 100 mL de 5,50-ditiobis (ácido 2-nitrobenzóico) (DTNB) 2,5 mM dissolvido em tampão de Tris-HCl 40 mM pH 8,9. Após 10 min em temperatura ambiente, a extinção das amostras foi medida a 412 nm no leitor de microplacas EZ READ 400. O branco consistiu em DTNB em vez de amostra; sua extinção foi retirada da amostra de teste antes combinando-o com a curva padrão (50–1000 μM). A Glutationa oxidada (GSSG) foi

testada com kit comercial, seguindo as instruções do fabricante (Sigma-AldrichTM, St. Luís, MO).

TAC foi determinada através do teste de redução do radical DPPH (2,2-diphenyl-1-picrylhydrazyl), segundo Janaszewsza; Bartosz (2002). Foram adicionados 20 µl de amostra, 480 µl de solução tampão de fosfato de sódio-potássio 10 mM (pH 7,4). Aos 500 µl de (amostra + tampão), foi adicionado 500 µl de solução 0,1 mM (metanol + de 2,2-difenil-1-picrilidrazilo). As amostras foram homogeneizadas e incubadas no escuro à temperatura ambiente durante 30 min. Em seguida centrifugadas durante 3 min a 700xg por 5 min e a absorvância foi lida a 520nm.

A concentração de ácido úrico (UA) foi determinada por ensaio colorimétrico (BioclinTM; Minas Gerais, Brasil). Os resultados foram expressos em mg/dL.

As substâncias reativas ao ácido tiobarbitúrico (TBARs) foram ensaiadas com 100 µL de saliva misturada com 500 µL de TCA 35% e 500 µL de Tris-HCl (200 mM, pH 7,4) e incubados por 10 min em temperatura ambiente. Um mL de Na2SO4 2 M e solução de ácido tiobarbitúrico 55 mM foram adicionadas e as amostras foram incubadas em 95°C por 45 min. As amostras foram resfriadas em gelo por 5 min e misturados após a adição de 1 mL de TCA 70%. Finalmente, as amostras foram centrifugadas a 15.000 g por 3 min e a absorbância do sobrenadante foi lida a 530 nm. Um coeficiente de extinção molar foi usado (ε = 1,56 x 105 M-1 cm-1) e os resultados foram expressos em nmol/mL.

A concentração de proteína total foi mensurada por analisador automático e detectada pelo método do biureto, usando BSA como padrão, conforme descrito por Gornall *et al.* (1949). O lactato foi medido usando um kit comercial (Bioclin^{TM}; Minas Gerais, Brasil). 2 mL de saliva foram misturados com 0,2 mL de tampão contendo lactato desidrogenase e NADp. A reação foi incubada por 5 minutos a 37 °C e lido a 340 nm. A curva padrão foi construída usando os reagentes do kit e os resultados foram expressos em g/dL.

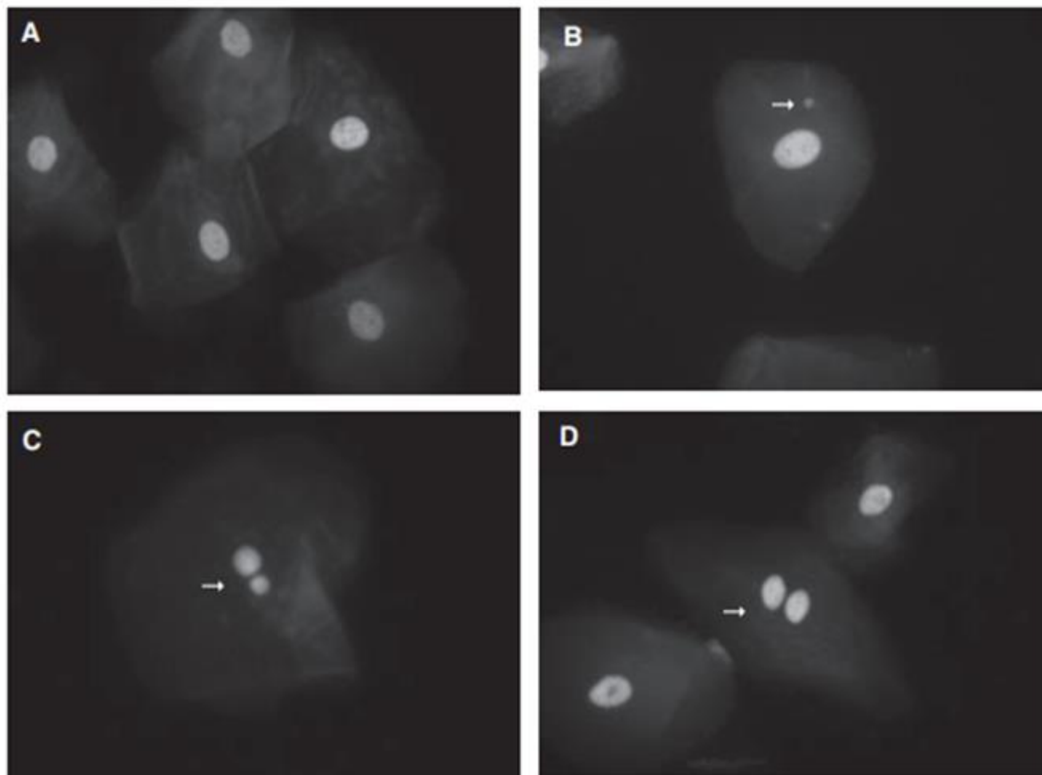
4.6 Ensaio de citoma de micronúcleos bucais e detecção de anormalidades nucleares

Foi solicitado aos indivíduos que enxaguassem a boca com água destilada e em seguida com clorexidina a 0,12%. Para a coleta de células utilizou-se uma lâmina para microscopia diretamente nas mucosas jugal direita e esquerda e a amostra foi espalhada

por uma segunda lâmina também pré-limpa e pré-codificada. Os esfregaços foram secos ao ar e fixados com álcool etílico absoluto PA por 2 H e depois corados com laranja de acridina (CAS nº 10127023; Sigma-Aldrich, St. Louis, MO, EUA) (adaptado de BONASSI *et al.,* 2009).

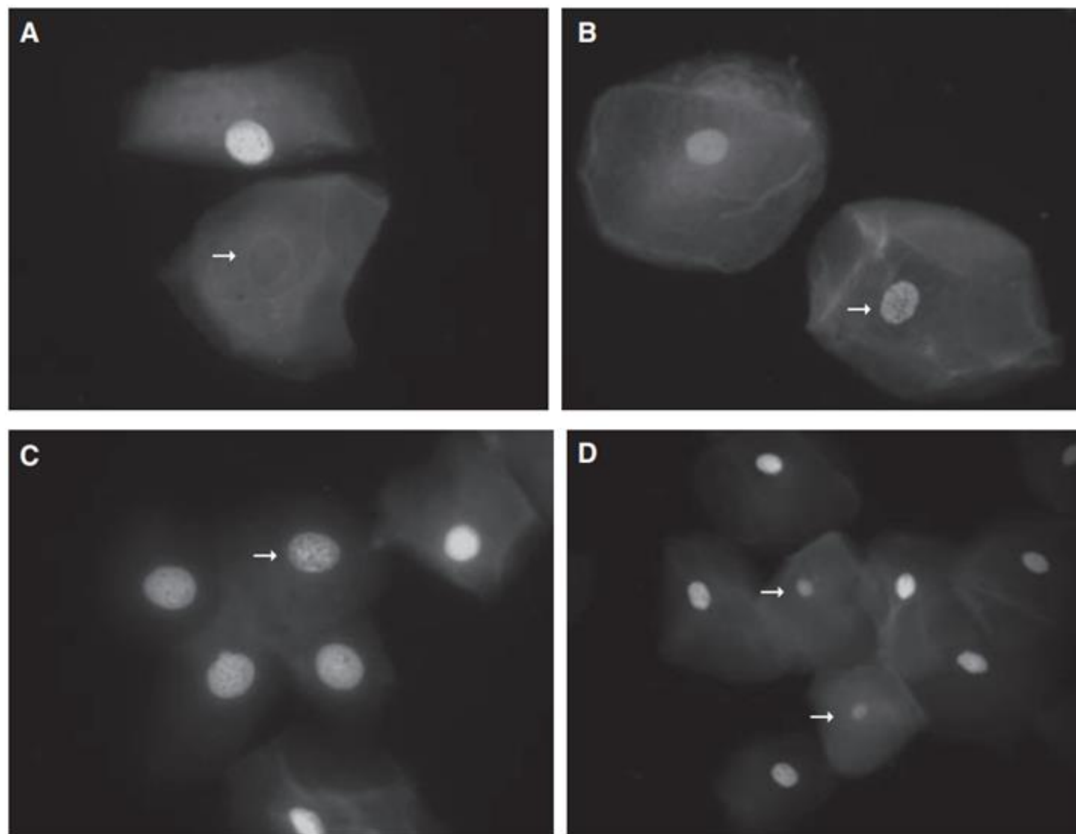
As lâminas pré-codificadas foram examinadas por um leitor, que contou cegamente os MNs e NA: células binucleadas, células com brotos nucleares, células cariolíticas, cariorréticas, cromatina condensada e picnóticas (ZAMORA-PEREZ *et al.*, 2014), conforme figuras 4 e 5, onde células aglomeradas não eram contabilizadas. Os critérios utilizados para a pontuação de MN e NA estão de acordo com os descritos por Thomas *et al.* (2009), e o número de células com MN e NA foram avaliados entre 500 células usando um microscópio Zeiss Axioimager D2 equipado com microscopia de fluorescência. Para captura da imagem em fluorescência utilizou-se um Zeiss filter set 00 (488000-0000-000) – excitação: BP 530/585 nm e emissão: LP 615 nm.

Figura 5. Imagens de microscopia óptica de fluorescência de células epiteliais da mucosa oral com diferentes danos ao DNA ou marcadores de genotoxicidade. (A) células normais; (B) Mincronúcleo; (C) Broto nuclear; (D) Célula binucleada



Fonte: Zamora-Perez *et al*., 2014.

Figura 6. Imagens de microscopia óptica de fluorescência que exemplificam morte celular ou marcadores de citotoxicidade das células da mucosa oral. (A) Cariolítica; (B) Cariorrética; (C) Cromatina condensada; (D) Célula picnótica



Fonte: Zamora-Perez *et al*., 2014.

4.7 Exames laboratoriais

Para confirmação da condição sistêmica dos pacientes, amostras de sangue foram coletadas após jejum noturno de 8 horas para a realização da análise do hemograma completo, lipidograma, glicemia em jejum, HbA1C e UA. As concentrações de colesterol HDL, LDL e de VLDL foram calculadas com base na equação de Friedewald *et al.* (1972). Esses exames são periodicamente realizados pelos pacientes que fazem tratamento na Clínica Médica do Hospital Universitário Clementino Fraga Filho da Universidade Federal do Rio de Janeiro, RJ, Brasil, no setor de Nutrologia e endocrinologia. Os pacientes dos demais grupos da pesquisa realizaram os exames em laboratórios que prestam serviço no estado do Rio de Janeiro.

4.8 Análise Estatística

Foi realizado o teste de Kolmogorov-Smirnov para avaliar se os dados apresentavam distribuição normal. Como não apresentaram distribuição normal, utilizou-se os testes não-paramétricos Kruskal-Wallis, Mann-Whitney e Qui-quadrado para avaliar as diferenças significativas das variáveis entre os grupos estudados. As correlações entre as variáveis numéricas foram calculadas com o coeficiente de correlação de Spermann (rho) e por análise de regressão linear, usando o método *stepwise,* e análise de regressão logística multivariada (método *forward Wald*). Apenas variáveis com significância de $p<0.05$ no modelo univariado foram incluídas na análise multivariada para cálculo de razão de chances (*odds ratio,* OR) e intervalo de confiança de 95%. Todas as análises foram realizadas em pacote estatístico (SPSS v22.0, Chicago, IL, USA). O nível de significância adotado foi de 5%.

5. MANUSCRITO I*

OXIDATIVE STRESS IN TYPE 2 DIABETES AND PERIODONTITIS: A SYSTEMATIC REVIEW*

Running Title: OXIDATIVE STRESS, T2DM & PERIODONTITIS

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Abstract

Oxidative stress is negatively influenced by type 2 diabetes mellitus and when associated with periodontitis the damage is greater. The authors' aim in this systematic review was to verify the scientific evidence for difference of oxidative stress biomarkers in individuals with type 2 diabetes mellitus with and without periodontitis. Observational studies, baseline data of prospective and interventional studies were searched through six databases and others sources. The quality assessment and the certainty of the evidence of the included studies were evaluated. Of 689 relevant articles, the authors included 9 studies for the final analysis. Among those studies, 4 cross-sectional, 3 case-control, and 2 interventional studies were included. The studies reported high concentrations of oxidizing agents and low antioxidants levels in individuals with type 2 diabetes mellitus and periodontitis when compared to with no periodontitis. Due to the high heterogeneity, methodological flaws, and low certainty of the evidence among included studies, it was not possible to determine whether there are or not differences in the oxidative stress levels in individuals with T2DM associated with periodontitis, and therefore, further prospective observational and interventional studies are recommended.

Keywords: diabetes, periodontal medicine, oxidative stress, systematic reviews and evidence-based medicine, inflammation.

Introduction

Type 2 diabetes mellitus (T2DM) is a significant risk factor for periodontitis and glycemic imbalance is the determining factor for bone loss that increases the risk and severity of periodontal disease (Preshaw *et al.*, 2012). The altered immunological response in T2DM influences periodontal diseases by altering the inflammatory responses, and periodontal wound healing. Additionally, it promotes the accumulation of advanced glycation end products (AGEs) that induce high levels of pro-inflammatory cytokines, and causes oxidative stress (OS) (Mealey, 2006).

Oxidative stress occurs when, due to a higher concentration of reactive oxygen species (ROS) or a decrease in antioxidant capacity, there is a lack of homeostasis (Pisoschi and Pop, 2015). This can occur in several systemic conditions. According to the literature, the tissue destruction that occurs in periodontal diseases can be caused by the high production of ROS by inflammatory cells, leading to oxidative stress (Gumus *et al.*, 2016).

The increase in ROS of local and systemic forms found in these pathologies can significantly affect the functioning pattern of several tissues and is closely related to the comorbidities associated with T2DM (Gumus *et al.*, 2016; Turrens, 2003). The increase in oxidative damage (Sezer *et al.*, 2012; Silva *et al.*, 2015) and lower levels of antioxidants (Baltacıoğlu *et al.*, 2014; D'Aiuto *et al.*, 2010) were found in patients with periodontitis compared to periodontally healthy individuals. The periodontal inflammatory condition can be explained by the local and systemic concentrations of some OS biomarkers (Silva *et al.*, 2015).

Periodontitis and T2DM are diseases with characteristics of chronic inflammation and a permanent increase in OS (Preshaw *et al.*, 2012; Silva *et al.*, 2015). However, no systematic review has assessed the level of OS biomarkers in type 2 diabetic individuals with and without periodontitis. Therefore, the aim of this systematic review was to answer the following question: Is there a difference in the level of OS in individuals with T2DM with and without periodontitis?

Methods

Registration and Protocol

This review was registered in the PROSPERO database under the protocol (https://www.crd.york.ac.uk/PROSPERO) ID: CRD42020190010, on August 7th, 2020 and was reported in accordance with the PRISMA checklist of systematic reviews and meta-analyses (Page *et al.*, 2021).

Eligibility Criteria

The eligibility criteria were defined based on the PECO research strategy as follows:

1. Participants (P): T2DM individuals of both genders and without distinction in age and ethnicity. Studies including individuals with the following conditions were excluded: chronic kidney disease, malignant neoplasms, AIDS (Acquired Immunodeficiency Syndrome), pregnant or lactating women, immunosuppression by medication, autoimmune diseases, evidence of other systemic diseases ASA III and IV, smokers or former-smokers for less than twenty years, users of orthodontic appliances, history of use of steroidal or non-steroidal anti-inflammatory drugs in the last three months prior to the study and during research, use of antibiotics in the last six months prior to the study and during research, as well as individuals who underwent supra and subgingival scaling and/or periodontal surgery in the last 6 months before the investigation.

2. Exposure (E): Presence of Periodontitis. Selection criteria established: Probing depth (PD) ≥ 4mm, Clinical attachment level (CAL) ≥ 4mm, presence of biofilm and bleeding on probing (BOP) > 10%. The examination should be performed by calibrated examiners, performing a full-mouth periodontal examination (six sites per tooth), using the North Carolina periodontal probe.

3. Comparison (C): Absence of Periodontitis. Selection criteria established: PD ≤ 3mm, CAL ≤ 3mm, little or no biofilm, and BOP <10%. The examination should be performed by one or more calibrated examiners, performing a full-mouth periodontal examination (six sites per tooth), using the North Carolina periodontal probe.

4. Outcome (O): OS levels for different parameters, such as AGEs, calcium, catalase (CAT), glutathione reductase (GRd), iron, magnesium, malondialdehyde (MDA),

nitric oxide (NO), oxidative stress index (OSI), protein carbonyl (Protein CO), small molecular antioxidant capacity (SMAC), superoxide dismutase (SOD), total antioxidant capacity (TAC), total oxidant status (TOS), vitamin C, zinc. If the outcomes of interest were not measured or not reported the studies were considered ineligible.

Information Sources

A systematic search of the literature was conducted on the following electronic databases: MEDLINE using the PubMed, Scopus, Embase, Web of Science, Cochrane Library and Virtual Health Library (VHL). Other sources were consulted through OpenGrey and Google Scholar. The electronic search was initially performed in June 2020, and database alerts with the search strategy were created in each database and were set to retrieve newly published articles until November 2022.

Literature Search Strategy

The search strategy included Medical Subject Headings (MeSH) terms, entry terms, free terms and keywords related to the aim of this review. No restrictions were placed on publication date or language. The strategy was developed using the boolean operators AND/OR, Medical Subject Headings (MeSH) terms, keywords, and other free terms related to "Diabetes mellitus, non-insulin dependent"; "Periodontitis, Periodontal diseases"; and "Oxidative stress, Antioxidants, Free radicals, Oxidants". The strategy was first idealized for Pubmed search engine use and then, adapted to each database according to their syntaxes rules. Specific search strategies were developed for each database (Supplementary material - Supplementary Table 1) with no restrictions on language or date. In addition, filters regarding VHL (virtual health library) database to LILACS and BBO collections were applied. A manual search was performed on the reference lists of the selected articles. Experts in the field were identified in the Scopus database by the "Analyze results tool" and contacted for ongoing studies or unpublished results regarding the focused question, using e-mail contact for up to five attempts. The search strategy was guided by an expert librarian (D.M.).

Articles from Google Scholar covered the first 100 matches and were then manually processed to check if possible eligible papers were missed from the main database search engines. When necessary, articles published in languages other than

English, Spanish, and Portuguese were translated using the Google® Translate Tool at https://translate.google.com.

Selection Process

Observational studies and baseline data of prospective/interventional studies, with the variables of interest, were included. Reviews, case reports, case series, expert opinions, and animal studies were excluded.

The retrieved articles were exported to Endnote® Web (www.myendnoteweb.com) to list, organize and remove duplicates. Articles retrieved from more than one database were computed only once. Authors and co-authors of studies that were not retrieved in the full text were contacted by e-mail up to five attempts, from June 2020 to November 2022.

All titles, abstracts, and full-text reading of the articles were independently analyzed by two reviewers (W.J.M.L. and C.C.M.) to determine whether they met the eligibility criteria. Whenever differences occurred between them, a consensus should be reached. When a study of interest had no abstract available, the study had its full text assessed for eligibility decision. Articles that did not meet the eligibility criteria were excluded at this stage. Selected articles were read in full to confirm eligibility. If a study had a sample overlapping with other studies and the same methodology criteria assessed, the least complete study was excluded. Whenever the two reviewers were unsure about the inclusion/exclusion of any publication, a discussion with a third reviewer (L.C.M.) was taken to solve any disagreement. Reasons for exclusion of articles after full-text examination were registered.

Data collection process/ data items

Data extraction regarding authors, year and country of the study, characteristics of participants (sample size, sample age, and T2DM duration in years), exposure (diagnostic criteria for periodontitis), body fluid collected (serum, saliva, and gingival crevicular fluid), assessment methods to oxidative stress parameters and statistics outcomes of interest (inferential and descriptive data) were extracted independently by 2 researchers (W.J.M.L. and C.C.M.). Any differences between the two examiners were solved by a third investigator (L.C.M.).

When missing data were detected, the corresponding author was contacted through electronic mail for up five consecutive weeks. In case there were no return from the authors to identify data in graphs, it was used the digital program WebPlotDigitizer online (https://automeris.io/WebPlotDigitizer/). The accuracy of extracted data was confirmed by another author.

Quality Assessment

The internal validity of the included studies was evaluated according to adaptations of Fowkes and Fulton's (1991) critical appraisal of published research guidelines (Supplementary material - Supplementary Table 2).

Effect measure

Due to the variability of the sample size between the studies, an adaptation was performed to determine the central value and variance of the samples. For outcomes, mean and standard deviation were used.

Synthesis methods and certainty of evidence assessment

Narrative syntheses were conducted for the results reported on each oxidative stress parameter. The certainty of evidence was determined using the Grading of Recommendations, Assessment, Development, and Evaluation Pro software (GRADEpro Guideline Development Tool) for the synthesized results on each OS parameter (Gradepro, 2015). The risk of bias, inconsistency, indirectness, suspicion of publication bias, presence of a large effect, dose-response gradient, and plausible confounders were the items considered to rate the overall certainty of evidence (Balshem *et al.*, 2011; Schünemann *et al.*, 2013). All the judgments were adapted to qualify the evidence synthesized in a narrative way (Murad *et al.*, 2017).

Results

Study Selection

A total of 689 studies were identified and retrieved: 664 from the database search, 3 from the alerts, and 22 from other sources. The main databases used as sources for studies were VHL (n=202) and Web of Science (n=144), followed by PubMed (n=132),

Embase (n=80), Scopus (n=70), Cochrane Library (n=39), other sources (n=0), and additional records identified through Google Scholar (n=22). The 140 duplicated titles/abstracts were eliminated through EndNote Software, and 53 through manual exclusion. All titles and abstracts (n=494) were analyzed and 463 were excluded according to the study criteria, resulting in 31 remaining studies, which were assessed for eligibility. Twenty-two were excluded due to the following reasons: absence of group T2DM without Periodontitis (n= 20), research participants with more than one systemic disease (n= 1), and an article that contained a part of an included study in this systematic review (n= 1) (Supplementary material - Supplementary Table 3).

Regarding the records identified via other resources, the first one hundred matches from the 30480 results in Google Scholar were selected for the study, and none were recorded in Opengrey. There were found 22 duplicate records, which were manually removed, and another 78 records were excluded after title/abstract reading. Alerts were set by November 2022 in Databases, and three articles were retrieved. However, they did not meet the inclusion criteria for eligibility. The flowchart of the study selection process is shown in Figure 1. A total of 9 articles were screened for the final analysis.

Study characteristics

The included studies were conducted in institutions such as universities, clinical centers, or hospitals in three different countries (Appendix - Table 1). Among those studies, 4 cross-sectional (Allen *et al.*, 2011; Pushparani *et al*., 2013; Pushparani, 2015; Takeda *et al*., 2006), 3 case-control (Pendyala *et al.*,2013; Trivedi *et al.*, 2013; Vincent *et al.*, 2018), and 2 interventional (Latha *et al.*, 2018; Shetty *et al.*, 2016) studies were included. In all studies, low levels of antioxidants were evaluated as primary outcome. Studies varied greatly regarding age, ranging from 20 years (Trivedi *et al.*, 2014) to 71 years (Latha *et al.*, 2018).

Periodontal parameters, plaque index (PI), BOP, PD, and CAL, were reported for periodontitis diagnosis, represented as mean and standard deviation (Latha *et al*. 2018; Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Shetty *et al.*, 2016; Takeda *et al.*, 2006; Trivedi *et al.*, 2014; Vincent *et al.*, 2018) or by mean percentage (Allen *et al.*, 2011; Pendyala *et al.*, 2013). The patients in the included studies had chronic periodontitis, according to 1999 AAP Classification (Artimage, 1999). The minimum number of teeth

of the research participants in the included studies ranged from 10 (Takeda *et al.*, 2006) to 20 functional teeth (Latha *et al*. 2018; Shetty *et al.*, 2016; Vincent *et al.*, 2018).

Data on T2DM duration was introduced in years and expressed in mean and standard deviation. Most studies included participants diagnosed with T2DM for at least 5 years (Allen *et al.*, 2011; Latha *et al*. 2018; Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Trivedi *et al.*, 2014). A study included participants with a minimum of 8 years of diagnosis for T2DM (Takeda *et al.*, 2006), other with participants at least 6 months of diagnosis (Vincent *et al*., 2018), while two studies (Pendyala *et al.*, 2013; Shetty *et al.*, 2016) did not report on this data.

The OS biomarkers were analyzed in more than one body fluid, including serum (Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Shetty *et al.*, 2016; Takeda *et al.*, 2006; Trivedi *et al.*, 2014), saliva (Latha *et al*. 2018; Pendyala *et al*., 2013; Trivedi *et al.*, 2014) and gingival crevicular fluid (GCF) (Vincent *et al*., 2018), and were expressed in mean and standard deviation, through different methods, comparing gold-standard or not. None of those studies directly cross-compared biomarkers findings between those body fluids.

Among the OS biomarkers studied a large number of antioxidants were evaluated such as SMAC (Allen *et al.*, 2011), TAC (Vincent *et al*., 2018; Pendyala *et al*., 2013), vitamin C (Pushpa Rani *et al.*, 2013), zinc (Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015), calcium (Pushpa Rani, 2015), magnesium (Pushpa Rani, 2015; Shetty *et al.*, 2016), CAT, SOD and GRd (Trivedi *et al.*, 2014). In addition, two oxidant agents were evaluated: NO (Latha *et al.*, 2018), and iron (Pushpa Rani, 2015). Furthermore, biomarkers of cell damage due to OS were also evaluated, such as Protein CO (Allen *et al.*, 2011), AGEs (Takeda *et al.*, 2006), MDA (Latha *et al.*, 2018; Trivedi *et al.*, 2014), TOS and OSI (Vincent *et al*., 2018).

Only one study (Trivedi *et al.*, 2014) evaluated the same biomarkers (MDA, SOD, CAT, and GRd) in blood and saliva, although no comparisons between findings from both fluids were compared, as mentioned before.

Quality Assessment

For the quality assessment, the included studies were classified according to the risk of bias, confounding factors, and chance. Susceptibility to bias and results occurred

by chance were observed in all analyzed studies (Allen *et al.*, 2011; Latha *et al.*, 2018; Pendyala *et al.*, 2013; Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Shetty *et al.*, 2016; Takeda *et al.*, 2006; Trivedi *et al.*, 2014; Vincent *et al.*, 2018). Confounding factors were observed in 3 out of the 9 analyzed studies (Appendix - Table 2) (Allen *et al.*, 2011; Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015). According with the criteria used, there was no sound study.

Results of individual studies and Synthesis

Some of the included studies demonstrated a significant correlation between high levels of oxidizing compounds and individuals with T2DM and periodontitis (Allen *et al.*, 2011; Latha *et al.*, 2018; Pushpa Rani, 2015; Takeda *et al.*, 2006). On the other hand, other studies presented a decrease in the levels of antioxidants in T2DM with periodontitis, when compared to T2DM without periodontitis (Allen *et al.*, 2011; Pendyala *et al.*, 2013; Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Shetty *et al.*, 2016; Takeda *et al.*, 2006; Trivedi *et al.*, 2014; Vincent *et al.*, 2018). Overall, the studies have shown that individuals with T2DM presented high OS levels, which are greater when associated with periodontitis.

Oxidants

In individuals with T2DM and periodontitis, Protein CO levels were higher compared with those without periodontitis. The same result of high levels was expressed for free radical damage markers (MDA, TOS, and OSI) in T2DM with periodontitis individuals when compared to T2DM individuals without periodontitis (Latha *et al.*, 2018; Trivedi *et al.*, 2014; Vincent *et al.*, 2018). In T2DM with periodontitis individuals, the NO levels were higher than in T2DM without periodontitis (Latha *et al.*, 2018). Pushpa Rani (2015) shown that elevated calcium and iron levels may be a contributing factor in many inflammatory conditions in T2DM with periodontitis individuals.

Antioxidants

The included studies demonstrated that some antioxidants (SMAC, TAC, Vitamin C, Zinc, Magnesium, CAT, SOD, and GRd) were detected in low concentrations in individuals with T2DM with no periodontitis. Additionally, those levels were even lower in individuals with T2DM with periodontitis (Allen *et al.*, 2011; Pendyala *et al.*, 2013;

Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015; Shetty *et al.*, 2016; Trivedi *et al.*, 2014; Vincent *et al.*, 2018).

Due to the variation in the study results and clinical/methodological heterogeneity, a meta-analysis was not appropriate.

Certainty of evidence

The certainty of the evidence was rated as very low for all the syntheses. The risk of bias affected the evidence because the included studies had important methodological limitations that could have altered the results. The evidence on the outcomes of MDA and TAC was inconsistent since there was variation in the reported effects by the studies. The item imprecision was also affected due to the reduced number of individuals included in the syntheses (less than the threshold of 400 recommended by GRADE) (Appendix - Table 3). The certainty of the evidence for the outcomes that included a single study (SMAC, Protein carbonyl, NO, Vitamin C, Zinc, Ca, Iron, AGEs, SOD, CAT, GRd, TOS, OSI) was lowered due to the risk of bias and impression (insufficient number of individuals). Publication bias was considered unsuspected, and since the evidence was affected by some of the previously mentioned criteria, no item was considered to raise the certainty. The judgments issued for the evaluation of the certainty of the evidence for the outcomes involving more than one study are presented in Appendix - Table 2.

Discussion

This systematic review aimed to synthesize the scientific evidence of studies on the association of OS biomarkers in individuals with T2DM with and without periodontitis. This systematic review was based on 9 not-sound studies and indicates that high levels of oxidizing agents and low concentration of antioxidants in T2DM are related with the presence of periodontitis when compared to individuals without periodontitis. To our knowledge, this is the first systematic review with quality assessment on this topic.

After filing in PROSPERO, studies selection, and full reading, it was observed that some studies had diagnostic criteria for periodontitis of CAL ≥3mm (Artimage, 1999) and, if they were excluded, some important data would be missing for the systematic review. Based on the new Classification of Periodontal Diseases and Conditions (Papapanou *et al.*, 2018), taking into consideration CAL, periodontitis is characterized

by: CAL ≥3mm in buccal or lingual/palatal in at least 2 teeth, without it being due to: 1) traumatic gingival recession; 2) dental caries extending to the cervical area of the tooth; 3) presence of insertion loss on the distal face of a second molar and associated with poor positioning or extraction of the third molar; 4) endoperiodontal lesion draining through the marginal periodontium; or 5) occurrence of vertical root fracture. Thus, this parameter had to be changed for articles selection.

Data show that patients with T2DM and periodontitis have impaired glycemic status and exhibit significantly lowered B-cell function and higher levels of HbA1c and fasting glucose than matched patients without periodontitis (Allen *et al.*, 2011). Those chronic inflammatory conditions are generally thought to be associated with increased OS with phagocytes, particularly neutrophils. Those cells are implicated in periodontal disease pathogenesis as they induce the generation of an oxidative burst during phagocytosis and killing (Pendyala *et al.*, 2013). On the other hand, ROS are associated with microvascular complications of T2DM. It is also known that severe periodontal disease can lead to endothelial dysfunction, which justify the assessment of effect of coexisting T2DM and periodontitis on the levels of OS markers (Latha *et al.*, 2018).

Periodontitis increases plasma biomarkers of OS as evidenced by the finding of reduced SMAC combined with increased levels of protein CO, which is a marker of protein oxidation. In the co-occurrence of T2DM and periodontitis, there is higher alterations in levels of these OS biomarkers. This suggests signs of enhanced OS in serum of T2DM with periodontitis individuals, showing that periodontitis has a negative effect on the already compromised oxidative status of T2DM patients (Allen *et al.*, 2011). Vincent *et al*. (2018) shown high levels of TOS in T2DM with periodontitis group when compared to periodontitis without T2DM encouraging the aforementioned data.

Role of nitric oxide system in T2DM is few studied and controversial to scientifics research, demonstrating higher level, low level, or no change. In chronic inflammatory processes, where activity of PMNs, macrophages, and endothelial cells is elevated, such as in periodontitis, the expression of iNOS is increased. The positive relationship between iNOS, inflammatory cytokines and other mediators reveals an immune-activated state due higher NO production. Thus, it might be higher in individuals with T2DM and chronic periodontitis when compared to without periodontitis cells (Latha *et al.*, 2018).

MDA is the major and commonly studied product of polyunsaturated fatty acid peroxidation that is shown to rise following OS. Glycated collagen has been shown a role in lipid glycoxidation compared to normal collagen that results in increased MDA in serum and tissues of diabetic subjects (Latha *et al.*, 2018). In T2DM individuals with periodontitis, MDA was reported to be higher when compared to individuals without periodontitis (Trivedi *et al.*, 2014).

It was reported that TAC levels in GCF was lower in patients with T2DM and periodontitis, compared to individual with only T2DM. The decrease in TAC levels reported could be attributed to elevated ROS levels that must be neutralize causing depletion of antioxidants, in addition to presence hyperglycemia, which is the primary cause of inflammation in T2DM individuals (Vincent *et al.*, 2018). In another study, the comparison of the TAC in saliva revealed lower antioxidant levels in T2DM with periodontitis compared to non-diabetic individuals with periodontitis (Pendyala *et al.*, 2013).

Excessive glucose levels induce free radical production and enhance OS by increased formation of AGEs. These pathologic mechanisms in T2DM with the preexisting periodontal disease could be responsible for exacerbated periodontal destruction seen in diabetics (Vincent *et al.*, 2018). Takeda *et al.* (2006) reported that AGEs were significantly related to periodontal deterioration associated with T2DM, showing that their level may be a suitable biomarker to reflect periodontal status in those patients.

Micronutrients are regulated by homeostatic processes and function as antioxidant in the control of damage caused by ROS (Roohani *et al.*, 2013). Calcium and iron play an essential role in regeneration, for coping with OS and for an adequate immune response, but in higher levels of calcium and iron in the serum may promote the development and progression of oxidative stress (Pushpa Rani, 2015). It has been reported that the mean calcium level in T2DM with periodontitis individuals was significantly higher when compared to systemically and periodontally healthy individuals. It was also demonstrated that an increased serum iron levels in T2DM with periodontitis can act as pro-oxidant agents, which are responsible for the formation of ROS (Pushpa Rani, 2015). High levels of circulating iron measured by transferrin saturation are associated with

increased oxidative stress. However, its association with higher serum ferritin levels is uncertain (Romeu *et al.*, 2013).

Additionally, it was reported that T2DM with periodontitis had lower zinc levels than those individuals with T2DM without periodontitis (Pushpa Rani *et al.*, 2013; Pushpa Rani, 2015). Zinc deficiency promotes the activation of N-methyl-D-aspartate (NMDA) receptors, which increase the intracellular concentration of calcium. In conditions where zinc is deficient, the NADPH oxidase enzymes and nitric oxide synthase are activated, favoring the production of reactive species of oxygen and nitrogen (Marreiro *et al.*, 2017).

Vitamin C is an essential dietary nutrient required for many enzyme reactions. It is an electron donor, and this property might account for all its known functions, such as water-soluble antioxidant in humans and protects from oxidative damage under conditions of increasing oxygen concentrations and apoptosis (Sulaiman and Shehadeh, 2010). Pushpa Rani *et al.* (2013) suggests that decreased vitamin C levels are associated with an increased risk for the development of oxidative stress in type 2 diabetes mellitus with periodontitis, considering that the antioxidant activity of ascorbic acid involves transfer of hydrogen rather than an electron.

Magnesium it is a cofactor of several antioxidant enzymes, including SOD, one of the most important antioxidant enzymes (Morais *et al.*, 2016) and it has been demonstrated that magnesium supplement has a beneficial effect on periodontitis (Van der Velden *et al.*, 2011). Furthermore, low magnesium levels may favor the onset and progression of T2DM, frequently seen in these patients (Corica *et al.*, 2006; Pushpa Rani, 2015). Pushpa Rani (2015) demonstrated that magnesium mean levels in T2DM without periodontitis and non-T2DM with periodontitis individuals were greater than the ones found for T2DM with periodontitis individuals and those findings were corroborated in another investigation (Shetty *et al.*, 2016).

The enzyme SOD is a key antioxidant that catalyzes the dismutation of superoxide (O_2^-), generating hydrogen peroxide (H_2O_2). The products CAT, GRd and GPx accelerate H_2O_2 reduction in water (Sies, 2015). Trivedi *et al.* (2014) demonstrated that SOD, CAT and GRd activities were higher in T2DM with periodontitis group compared with T2DM without periodontitis group. In periodontitis, SOD, CAT and GRd levels are low due

antioxidant depletion, to ongoing free radical activity and destruction of protective antioxidant species.

Clinical investigations must always focus on correct matching for gender, age, and periodontal status to reduce potential confounding factors. Latha *et al.* (2018), Pendyala *et al.* (2013), Takeda *et al.* (2006) and Trivedi *et al.* (2014) follow these restrictions and the distribution of age and gender was similar between groups, suggesting that they were well matched. Thus, their results might present greater validity and relatively fewer confounding factors.

The analysis of non-randomized clinical trials properly reported most of the criteria analyzed in Summary questions (Bias, Confounding and Chance) as present in 3 studies. In six studies (Latha *et al.*, 2018; Pendyala *et al.*, 2013; Shetty *et al.*, 2016; Takeda *et al.*, 2006; Trivedi *et al.*, 2014; Vincent *et al.*, 2018) confounding factors were no detected. Shetty *et al.* (2016) defined their study as a randomized clinical trial, but it has four groups (i.e., T2DM with periodontitis, T2DM without Periodontitis, non-T2DM with periodontitis, and systemically and periodontally healthy) with distinct interventions (scaling and root planning, and oral hygiene instructions), and there is no random allocation of groups/ treatment. In summary, their results are inconsistent and the evidence regarding OS biomarkers, T2DM and periodontitis relationship is lacking strong evidence.

Of the nine included studies, three assessed biomarkers in saliva and this suggests its potential relevance as an important alternative biological fluid. Included studies in this systematic review are following the expected direction of the association, that there is difference in OS levels between T2DM individuals with and without periodontitis. These studies reported the relationship between high levels of oxidizing compounds or decrease of antioxidants in T2DM with periodontitis individuals, when compared to T2DM without periodontitis individuals. Therefore, these data suggest that periodontitis has a negative influence on OS in T2DM and recognize that an early diagnosis of periodontitis may be important for prevention of a negative impact on the OS biomarkers.

However, considering the few studies found, the methodological flaws, few markers studied and absence homogeneity in the evaluation of redox balance markers, as well as, the very low certainty of the evidence among included studies, ie there was no

sound studies included in this systematic review, these results should be viewed with caution. Therefore, it was not possible to determine whether there are or not differences in the oxidative stress levels in individuals with T2DM associated with periodontitis and further prospective observational and interventional studies are recommended.

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Disclosure

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Supplementary Table 1 Electronic database and search strategy up to November 2022

PUBMED	(Diabetes Mellitus[Mesh] OR Diabetes Mellitus[Tiab] OR Diabetes Mellitus, Type 2[Mesh] OR Diabetes Mellitus Non Insulin Dependent[Tiab] OR Diabetes Mellitus Stable[Tiab] OR NIDDM[Tiab] OR MODY[Tiab] OR Maturity Onset Diabetes[Tiab] OR Type 2 Diabetes[Tiab] OR Diabetes Type 2[Tiab]) AND (Periodontal Diseases[Mesh] OR Periodontal Disease*[Tiab] OR Periodontitis[Mesh] OR Periodont*[Tiab] OR Aggressive Periodontitis[Mesh] OR Aggressive Periodont*[Tiab] OR Chronic Periodontitis[Mesh] OR Chronic Periodont*[Tiab]) AND (Oxidative Stress[Mesh] OR Oxidative Stress*[Tiab] OR Antioxidants[Mesh] OR Antioxidant*[Tiab] OR Antioxidant effect*[Tiab] OR Free radicals[Mesh] OR Free radicals[Tiab] OR Oxidants[Mesh] OR Oxidant*[Tiab] OR Oxidizing Agents[Tiab])
SCOPUS	TITLE-ABS-KEY(("Diabetes Mellitus" OR "Diabetes Mellitus, Type 2" OR "Diabetes Mellitus Non Insulin Dependent" OR "Diabetes Mellitus Stable" OR NIDDM OR MODY OR "Maturity Onset Diabetes" OR "Type 2 Diabetes" OR "Diabetes Type 2") AND (Periodontal Disease* OR Periodont* OR Aggressive Periodont* OR Chronic Periodont*) AND (Oxidative Stress* OR Antioxidant* OR Antioxidant effect* OR "Free radicals" OR Oxidant* OR "Oxidizing Agents"))
WEB OF SCIENCE	(("Diabetes Mellitus" OR "Diabetes Mellitus, Type 2" OR "Diabetes Mellitus Non Insulin Dependent" OR "Diabetes Mellitus Stable" OR NIDDM OR MODY OR "Maturity Onset Diabetes" OR "Type 2 Diabetes" OR "Diabetes Type 2") AND (Periodontal Disease* OR Periodont* OR Aggressive Periodont* OR Chronic Periodont*) AND (Oxidative Stress* OR Antioxidant* OR Antioxidant effect* OR "Free radicals" OR Oxidant* OR "Oxidizing Agents"))
COCHRANE	#1 MeSH descriptor: [Diabetes Mellitus] explode all trees #2 "Diabetes Mellitus" #3 MeSH descriptor: [Diabetes Mellitus, Type 2] explode all trees #4 ("Diabetes Mellitus Non Insulin Dependent" OR "Diabetes Mellitus Stable" OR NIDDM OR MODY OR "Maturity Onset Diabetes" OR "Type 2 Diabetes" OR "Diabetes Type 2") #5 #1 OR #2 OR #3 OR #4 #6 MeSH descriptor: [Periodontal Diseases] explode all trees #7 Periodontal disease* #8 MeSH descriptor: [Periodontitis] explode all trees #9 Periodont* #10 MeSH descriptor: [Aggressive Periodontitis] explode all trees #11 Aggressive Periodont* #12 MeSH descriptor: [Chronic Periodontitis] explode all trees #13 Chronic Periodont* #14 #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 #15 MeSH descriptor: [Oxidative Stress] explode all trees #16 Oxidative Stress*

	#17	MeSH descriptor: [Antioxidants] explode all trees
	#18	(Antioxidant* OR Antioxidant effect*)
	#19	MeSH descriptor: [Free Radicals] explode all trees
	#20	"Free radicals"
	#21	MeSH descriptor: [Oxidants] explode all trees
	#22	(Oxidant* OR "Oxidizing Agents")
	#23	#15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22
	#24	#5 AND #14 AND #23
LILACS and BBO	tw:(tw:((tw:((mh: "Diabetes Mellitus" OR mh: "Diabetes Mellitus, Type 2" OR "Diabetes Mellitus Tipo 2" OR "Diabetes Mellitus Non Insulin Dependent" OR "Diabetes Mellitus não dependente de insulina" OR "Diabetes Mellitus Stable" OR "Diabetes Mellitus estável" OR niddm OR dmndi OR mody OR "Maturity Onset Diabetes" OR "Diabetes com início na maturidade" OR "Type 2 Diabetes" OR "Diabetes tipo 2" OR "Diabetes Type 2" OR "Tipo 2 Diabetes"))) AND (tw:((mh: "Periodontal Diseases" OR "doenças periodontais" OR “periodontal disease” OR “periodontal diseases” OR "doença periodontal" OR "doenças periodontais" OR mh: periodontitis OR periodontite OR periodontitis OR periodontite OR mh: "Aggressive Periodontitis" OR "Periodontite Agressiva" OR "Aggressive Periodontitis" OR "Periodontite Agressiva" OR mh: "Chronic Periodontitis" OR "Periodontite Crônica" OR "Chronic Periodontitis" OR "Periodontite Crônica"))) AND (tw:((mh: "Oxidative Stress" OR "Estresse Oxidativo" OR oxidative stress* OR "Estresse Oxidativo" OR "Estresses oxidativos" OR mh: antioxidants OR antioxidantes OR antioxidant OR antioxidants OR antioxidante OR "antioxidant effect" OR "Antioxidants effects" OR "Efeito antioxidante" OR "Efeitos antioxidantes" OR mh: "Free radicals" OR "Radicais livres" OR "Free radicals" OR "Radicais livres" OR mh: oxidants OR oxidantes OR oxidants OR oxidant OR oxidante* OR "Oxidizing Agents" OR " agentes oxidantes")))) AND (db:("LILACS" OR "BBO"))) AND (type:("article"))	
EMBASE	('diabetes mellitus':ti,ab,kw OR 'diabetes mellitus, type 2':ti,ab,kw OR 'diabetes mellitus non insulin dependent':ti,ab,kw OR 'diabetes mellitus stable':ti,ab,kw OR niddm:ti,ab,kw OR mody:ti,ab,kw OR 'maturity onset diabetes':ti,ab,kw OR 'type 2 diabetes':ti,ab,kw OR 'diabetes type 2':ti,ab,kw) AND ('periodontal disease*':ti,ab,kw OR periodont*:ti,ab,kw OR 'aggressive periodont*':ti,ab,kw OR 'chronic periodont*':ti,ab,kw) AND ('oxidative stress*':ti,ab,kw OR antioxidant*:ti,ab,kw OR 'antioxidant effect*':ti,ab,kw OR 'free radicals':ti,ab,kw OR oxidant*:ti,ab,kw OR 'oxidizing agents':ti,ab,kw)	
OPEN GREY	Periodontitis and Diabetes and Antioxidants Periodontitis and Diabetes and Free radicals Periodontitis and Diabetes and Oxidants Periodontitis and Diabetes and Oxidative Stress Periodont* and Diabetes and Antioxidants Periodont* and Diabetes and Free radicals Periodont* and Diabetes and Oxidants Periodont* and Diabetes and Antioxidants Periodont* and Diabetes and Oxidative Stress	

	Periodont* and Diabetes and Antioxidant*
	Periodont* and Diabetes and Oxidative Stress*
	Periodontitis and Diabetes and Antioxidant*
	Periodontitis and Diabetes and Oxidative Stress*
GOOGLE SCHOLAR	Periodontitis and diabetes type 2 and oxidative stress
	Periodontite e diabetes tipo 2 e estresse oxidativo

Supplementary Table 2 Criteria adopted to risk of bias classification (adapted from Fowkes and Fulton, 1991)

Study design appropriate to objective?	Prevalence - Cross-sectional	0 – if the study, or part of study, included in the present systematic review reported oxidative stress in type 2 diabetics with periodontitis and had a cross-sectional design
	Prognosis – Cohort	0 – if the study, or part of study, included in the present systematic review reported oxidative stress in type 2 diabetics with periodontitis and had a Cohort design
	Treatment - Controlled trial	0 - if the study, or part of study, included in the present systematic review evaluate some type of treatment related periodontitis or type 2 diabetes mellitus and measurement oxidative stress levels and had a controlled trial design
	Cause - Cohort, case-control, cross-sectiona	0 - if the study, or part of study, included in the present systematic review evaluate some type of relationship / association / risk reported of oxidative stress alteration in type 2 diabetics with periodontitis had a cross-sectional, case-control or cohort design, respectively.
Study sample representative?	Source of sample	(0) case group was obtained from various referral hospital (multicentre). (+) case group was composed of individuals from a reference center or referral hospital. (++) case group was obtained from unspecified locations.
	Sampling method	(0) probabilistic sampling (simple random, stratified, blocks). (+) did not use any type of randomization, but authors of the present systematic review judged that could not influence in outcome evaluation, as all eligible participants from a specific site/place were included. (++) did not use any type of randomization, but authors of the present systematic review judged that could influence in outcome evaluation by using a convenience sample.
	Sample size	(0) sample size calculation was described considering all variables and study groups. (+) did not perform sample size calculation but had a representative sample, using a sample size equal or higher than 97 subjects (median of samples from included studies). (++) did not mention such sample size calculation or representative sample, using a sample size lower than 97 subjects (median of samples from included studies).
	Inclusion/exclusion criteria	(0) Selection criteria properly established. Periodontitis: PBS≥ 4mm, NCI≥ 4mm, presence of biofilm, bleeding on probing (> 10%) and presence of suppuration. Examination performed by a single calibrated examiner or more than one (calibrated), who underwent a complete periodontal examination (in the six sites of the teeth

		present), using a standard probe for the diagnosis of periodontitis (North Carolina 15mm). (+)Selection criteria established, but some criteria were not considered (do not mention one of the criteria for diagnosing periodontitis previously shown or the examiner is not calibrated). (++) Selection criteria loosely established or absence of two or more periodontitis selection criteria.
	Non-respondents	N. A. - the authors of the present systematic review judged that the non-respondent's rate could not influence in outcome evaluation.
Control group acceptable?	Definition of controls	(0) Selection criteria properly established. Absence of periodontitis: PBS≤ 3mm, NCI≤ 3mm, little or no biofilm, no or little bleeding on probing (<10%) and no suppuration. Examination performed by a single calibrated examiner or more than one calibrated, who underwent a complete periodontal examination (at the six sites of the teeth present), using a standard probe for the diagnosis of periodontitis (North Carolina 15mm). (+) Selection criteria established, but some criteria were not considered (do not mention one of the criteria for periodontal health shown previously). (++) Selection criteria loosely established. Absence of two or more selection criteria for periodontal health.
	Source of controls	(0) control group was obtained from various referral hospital (multicentre). (+) control group was composed of individuals from a reference center or referral hospital. (++) control group was obtained from unspecified locations.
	Matching/randomization	(0) group control was paired according to gender, age and duration of diabetes. (+) only matched by two of the criteria mentioned above. (++) control group was not paired.study mentioned that case and control groups were matching for sex, age and general health.
	Comparable characteristics	(0) There is no difference between the groups regarding age, gender and duration of diabetes. (+) the groups are different only in relation to two criteria. (++) the groups are different in relation to age or duration of diabetes.
Quality of measurements and outcomes?	Validity	(0) Use of the gold standard method for determining the biomarker (+) adequate test, but it is not the gold standard. (++) Test not suitable.
	Reproducibility	(0) An experienced and calibrated evaluator and that there was acceptable reproducibility in the measurement of oxidative stress markers. (+) a trained evaluator, but the study's reproducibility analysis was not performed.

		(++) an uncalibrated evaluator, but the study was not reproducible or was not even mentioned in the study.
	Blindness	(0) Researcher who collected and analyzed samples were blinded to the study group. (+) Researcher who collected or analyzed the samples was blinded to the study group. (++) There was no blinding in any phase of the study.
	Quality control	(0) Adequate acquisition, processing and storage of samples. Adequate description of the parameters used to measure oxidative stress. (+) one of the points described above was neglected or not mentioned. (++) two or more points described above were neglected or not mentioned.
Completeness?	Compliance	NA (question did not apply to study methodology)
	Dropouts	NA (question did not apply to study methodology)
	Deaths	NA (question did not apply to study methodology)
	Missing data	(0) The study reports the number of missing data (up to 30%) and the reasons. (or the absence of missing data). (+) the number of missing data is reported without explaining the reasons (with up to 30% of the missing data). (++) nothing is specified or there was a loss greater than 30%.
Distorting influences?	Extraneous treatments	NA (question did not apply to study methodology)
	Contamination	NA (question did not apply to study methodology)
	Changes over time	NA (question did not apply to study methodology)
	Confounding factors	(0) Presence of comorbidities: chronic kidney disease, high blood pressure, dyslipidemia, obesity and immunosuppressed by medication. Other metabolic changes. Age and gender. Duration of diabetes. (+) was assigned when 1 or 2 of these characteristics were present (++) if there were 3 or more.
	Distortion reduced by analysis	(0) Stratified data analysis was performed, or regression analysis considering possible confounding factors. Or, there was no need due to the absence of confounding factors. (+) adjustment of the analyzes was performed only in relation to some confounding factors. (++) there were confounding factors, but this was not considered in the analyzes.

NA – Not applied.

Summary Questions		
	Bias - Are the results erroneously biased in a certain direction?	Yes / No
	Confounding - Are there any serious confounding or other distorting influences?	Yes / No
	Chance - Is it likely that the results occurred by chance?	Yes / No

Supplementary Table 3 Studies excluded and reason of the exclusion

Reason 1. Absence of group T2DM without Periodontitis
PUBMED
1. Arana C, et al. Increased salivary oxidative stress parameters in patients with type 2 diabetes: Relation with periodontal disease. Endocrinol, Diabetes Nutr. 2017; 64 (5):258-264.
2. Bazyar H, et al. The Impacts of Synbiotic Supplementation on Periodontal Indices and Biomarkers of Oxidative Stress in Type 2 Diabetes Mellitus Patients with Chronic Periodontitis Under Non-Surgical Periodontal Therapy. A Double-Blind, Placebo-Controlled Trial. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2020; 13: 19-29.
3. Duarte PM, et al. The expression of antioxidant enzymes in the gingivae of type 2 diabetics with chronic periodontitis. Archives of oral biology 2012; 57: 161-168.
4. El-Sharkawy H, et al. Propolis Improves Periodontal Status and Glycemic Control in Subjects With Type 2 Diabetes Mellitus and Chronic Periodontitis: A Randomized Clinical Trial. Journal of Periodontology 2016; 87 (12):1418-1426.
5. Gayathri S, et al. Effect of Initial Periodontal Therapy on Serum Nitric Oxide Levels in Chronic Periodontitis Patients with or without Type 2 Diabetes Mellitus. The Journal of Contemporary Dental Practice 2019; 20 (2):197-203.
6. Javid AZ, et al. Impact of resveratrol supplementation on inflammatory, antioxidant, and periodontal markers in type 2 diabetic patients with chronic periodontitis. Diabetes & Metabolic Syndrome: Clinical Research & Reviews 2019; 13:2769-2774.
7. Javid AZ, et al. The effects of ginger supplementation on inflammatory, antioxidant, and periodontal parameters in type 2 diabetes mellitus patients with chronic periodontitis under non-surgical periodontal therapy. A double-blind, placebo-controlled trial. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2019; 12:1751-1761.
8. Koregol AC, et al. 8-Isoprostane in chronic periodontitis and type II diabetes: Exploring the link. Dental Research, Dental Clinics, Dental Prospects JODDD 2018; 12(4):252-257.
9. Koromantzos PA, et al. Effect of Non-Surgical Periodontal Therapy on C-Reactive Protein, Oxidative Stress, and Matrix Metalloproteinase (MMP)-9 and MMP-2 Levels in Patients With Type 2 Diabetes: A Randomized Controlled Study. Journal of Periodontology 2012; 83(1):1-10.

10. Masi S, et al. Mitochondrial oxidative stress, endothelial function and metabolic control in patients with type II diabetes and periodontitis: A randomized controlled clinical trial. International Journal of Cardiology 2017; 15 (271):263-268.

11. Mizuno H, et al. The effects of non-surgical periodontal treatment on glycemic control, oxidative stress balance and quality of life in patients

with type 2 diabetes: A randomized clinical trial. PLoS ONE 2017; 12(11):1-17.

12. Patil VS, et al. Chronic Periodontitis in Type 2 Diabetes Mellitus: Oxidative Stress as a Common Factor in Periodontal Tissue Injury. Journal of Clinical and Diagnostic Research 2016; 10(4):12-16.

13. Pradeep AR, et al. 4-Hydroxy-2-nonenal, an oxidative stress marker in crevicular fluid and serum in type 2 diabetes with chronic periodontitis. Contemp Clin Dent. 2013; 4(3):281-285.

14. Sonoki, K, et al. Decreased Lipid Peroxidation Following Periodontal Therapy in Type 2 Diabetic Patients. J Periodontol. 2006; 77(11):1907-1913.

15. Thomas B, et al. A comparative evaluation of antioxidant enzymes and selenium in the serum of periodontitis patients with diabetes mellitus type 2. Contemp Clin Dent. 2013; 4(2):176-180.

16. Thomas B, et al. Serum levels of antioxidants and superoxide dismutase in periodontitis patients with diabetes type 2. Journal of Indian Society of Periodontology 2014; 18(4):451-58.

17. Zhu C, et al. The therapeutic role of baicalein in combating experimental periodontitis with diabetes via Nrf2 antioxidant signaling pathway. J Periodont Res. 2020; 55(3):381-391.

COCHRANE

1. Gholinezhad H, et al. Using Ginger Supplement in Adjunct with Non-surgical Periodontal Therapy Improves Metabolic and Periodontal Parameters in Patients with Type 2 Diabetes Mellitus (DM) and Chronic Periodontitis. A Double-Blind, Placebo-Controlled Trial. Journal of Herbal Medicine 2019; 20(100315).

VHL

1. Navarro MLR, et al. Niveles de proteína carbonilada y capacidad antioxidante total en pacientes con diabetes mellitus tipo 2 de reciente diagnóstico y enfermedad periodontal. Revista ADM 2019; 76 (4): 208-213.

2. Penagos AGV, Núñez VMM. Relación del estrés oxidativo con la enfermedad periodontal en adultos mayores con diabetes mellitus tipo 2. Revista ADM 2006; 63 (5):189-194.
Reason 2. research participants with more than one systemic disease
WEB OF SCIENCE 1. Bastos AS. Lipid Peroxidation Is Associated with the Severity of Periodontal Disease and Local Inflammatory Markers in Patients with Type 2 Diabetes. J Clin Endocrinol Metab 2012; 97 (8):1353-1362.
Reason 3. summary study of a larger included in this systematic review
EMBASE 1. Pushparani DS. Serum zinc and iron level in type 2 diabetes mellitus with periodontitis. International Journal of PharmTech Research, 2015; 7 (1):165-171.

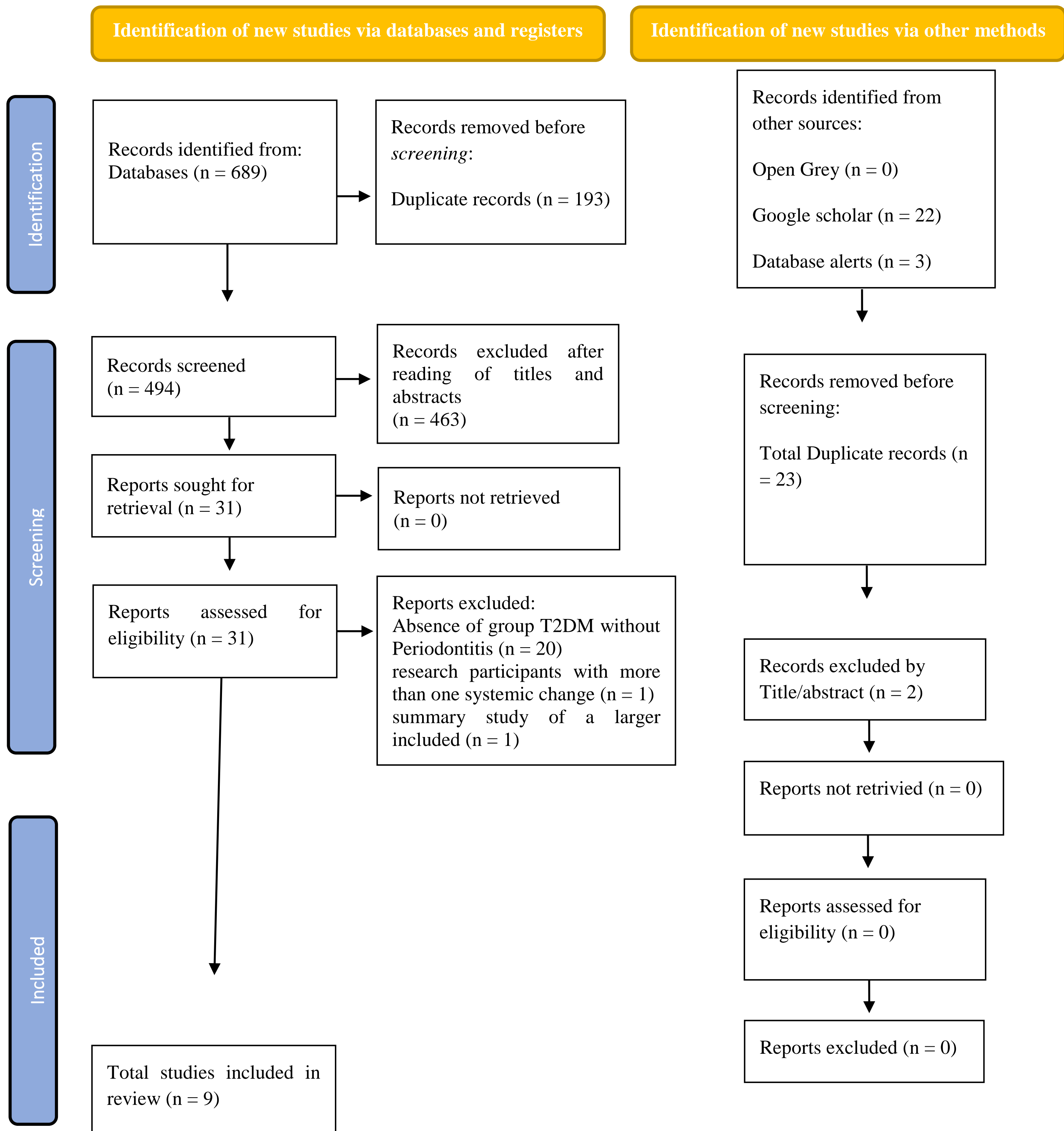

Figure 1. Flowchart diagram of literature search according to Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines, published in 2020.

Appendix - Table 1 Characteristics of the sample and data collection of the 9 included studies.

		Characteristics of Subjects			Exposure	Outcomes		Numeric Data	
Author et al. / year / Country	Study design	Sample size	Sample age (Mean±SD)	Diabetes duration in years (Mean±SD)	Diagnostic criteria for periodontitis	Body fluid	Oxidative Stress Parameters (Assessment method)	Oxidative stress markers (Mean±SD)	
								T2DM	T2DM + P
ALLEN et al. (2011) / Ireland	Cross-sectional	T2DM: 20 T2DM + P: 20	T2DM: 55±7 T2DM+P: 56±7	T2DM: 5.7±16.9 T2DM+P: 7.0±14.5 They should be on dose of oral hypoglycemic drugs, Anti-inflammatories, statins, ace inhibitors, β-blockers and diuretics.	- More than 16 teeth with periodontal pockets > 4mm in at least 6 sites. PD (mm) BOP (%) PI (%)	Serum	SMAC (Chemiluminescence) Protein CO (ELISA)	523.4±111.1 1.99±0.85	452.6±100.1 2.71±0.94
LATHA et al. (2018) / India	Intervention	T2DM: 10 T2DM + P: 15	T2DM: 56.8±8.71 T2DMP: 51.13±6.75	The subjects should have been diagnosed with type 2 diabetes for at least 5 years. They should be on stable dose of insulin/oral hypoglycemic drugs.	- More than 20 teeth with clinical attachment loss of ≥ 5mm in at least 30% of the sites. PI (Mean±SD) BOP (Mean±SD)	Saliva	MDA (spectrophotometric method) NO (spectrophotometric method)	2.19±1.62 1.76±1.02	2.17±0.52 9.08±2.33

					PD (Mean±SD) CAL (Mean±SD)				
PENDYALA et al. (2013) / India	Case-control	T2DM: 30 T2DM+ P: 30	40-65 years	Time of diagnosis for T2DM was not informed. These patients were not under any oral hypoglycemic agents and/or insulin therapy	- More than 14 teeth with two or more tooth sites with PD ≥ 4 mm or CAL of 4 mm that bled on probing. PD (mm) CAL (mm) PD ≥ 4mm (%) CAL ≥4mm (%) PI (%) BOP (%)	Saliva	TAC (spectrophotometric method)	1.24±0.18	0.40±0.09
PUSHPARANI et al. (2013) / India	Cross-sectional	T2DM: 150 T2DM + P: 150	46.26 ± 10.02 44.42 ± 10.37	Diagnosed by a physician by means of oral glucose tolerance test, for at least the past 5 years	- More than 30% of the sites with CAL ≥ 3 mm and PD ≥ 5 mm, at least 2 teeth in each quadrant with the condition of 20 teeth in all the subjects. CAL (mm)	Serum	Vitamin C (DNPH method) Zinc (Nitro-PAPS)	1.25±3.58 † 157.2±45.8	0.99±1.66 † 106.8±31.83

					PD (mm)				
PUSHPARANI et al. (2015) / India	Cross-sectional	T2DM: 150 T2DM + P: 150	46.26±10.02 44.42±10.37	Diagnosed by a physician by means of oral glucose tolerance test, for at least the past 5 years	- More than 30% of the sites with clinical attachment level (CAL) ≥ 3mm and probing depth (PD) ≥ 5 mm, at least 2 teeth in each quadrant with the condition of 20 teeth in all the subjects. CAL (mm) PD (mm)	Serum	Calcium (OCPC) Iron (Ramsay's dipyridyl method) Magnesium (Absorbance of 520 nm, with Xylidyl Blue dye reagent kit) Zinc (Nitro-PAPS)	8.59±0.86 76.53±20.23 1.56±0.42 157.2±45.8	11.79±2.07 114.9±40.91 1.45±0.41 106.8±31.83
SHETTY et al. (2016) / Índia	Intervention	T2DM: 30 T2DM+ P: 30	25-60 years	Time of diagnosis for T2DM was not informed.	- More than 20 teeth present. Used the classification of Löe (1967) to periodontal disease: gingival index, plaque index and retention index. GI (0, 1, 2 and 3)	Serum	Magnesium (semi-autoanalyzer)	1.01±0.28	0.92±0.23

					PI (0, 1, 2 and 3) Retentetion index system (0, 1, 2 and 3)				
TAKEDA et al. (2006) / Japan	Cross-sectional	T2DM: 28 T2DM+ P: 69	57.8± 12.1	T2DM: 8.0±7.7 T2DM+P: 8.6±7.6 The control with medicaments was not informed.	- Subjects had ≥ 10 functional teeth and more than one tooth with CAL > 5 mm. BOP (Presence or Ausence of bleeding within 5 to 20 seconds after probing) PD (mm) CAL (%)	Serum	AGEs (uninformed)	2.6±1.0	2.5±0.8
TRIVEDI et al. (2014) / Índia	Case-control	T2DM: 30 T2DM+ P: 30	20-65 years, categorizaded (≤40 years and >40 years)	T2DM: 4.33±3.44 T2DM+P: 5.53±4.05 Were controlled diabetics being treated with stable doses of oral hypoglycemic agents	- Patients with two or more tooth sites with probing depth ≥ 4mm or clinical attachment loss (CAL) ≥ 4mm that bled on probing. - Minimum number of teeth not informed.	Saliva	MDA (spectrophotometric method) SOD (Mc Cord and Fridovich)	1.91±1,.72 13.45±2.80	10.79±8.07 14.08±4.28

				and /or insulin by an endocrinologista.	PI (Presence or Ausence) GI (Presence or Ausence) PD (mm) CAL (mm)		CAT (Mc Cord and Fridovich)	0.04±0.04	0.04±0.03
							GRd (Mc Cord and Fridovich)	13.73±2.79	18.33±7.47
						Serum	MDA (spectrophotometric method)	13.01±5.55	15.91±6.98
							SOD (Mc Cord and Fridovich)	19.05±5.88	26.84±12.11
							CAT (Mc Cord and Fridovich)	0.06±0.03	0.06±0.03
							GRd (Mc Cord and Fridovich)	6,28±4,96	12,15±6,11
VINCENT et al. (2018) / India	Case-control	T2DM: 20 T2DM + P: 20	25-65 years	Participants diagnosed with type II DM by a diabetologist	- Minimum of 20 teeth present with at least 5 teeth in each	GCF	TAC (Erel O's novel automated method)	0.77±0.27	0.69±0.19

	and under treatment with oral hypoglycemic drugs and diet control for a minimum of 6 months were included	quadrant, with a probing depth (PD) of ≥5mm with clinical attachment loss of ≥1mm in more than 30% of sites with mild to moderate periodontitis, and presence of >30% of sites with bleeding on probing.	TOS (Erel O's novel automated method)	7.84±1.50	10.05±3.26
			OSI (Erel O's novel automated method)	1.00±0.52	1.39±0.62
		PI			
		BOP			
		PD (mm)			
		CAL (mm)			

Note: All patients in the diabetes group were diagnosed based on the criteria of the World Health Organization (Fasting glucose ≥126 mg/dL, HbA1c levels >5,6% and oral glucose tolerance test ≥200 mg/dL).

AGEs: Advanced Glycation End-products; BOP: Bleeding on probing; CAL: Clinical attachment loss; CAT: Catalase; DNPH: 2, 4-dinitrophenylhydrazine method; T2DM: Type 2 diabetes mellitus individuals; T2DM + P: Type 2 diabetes mellitus with periodontitis individuals; ELISA: Enzyme-Linked Immunosorbent Assay; GCF: Gingival crevicular fluid; GI: Gum Index; GRd: Glutathione reductase; HbA1c: Glycated hemoglobin; MDA: Malondialdehyde; Nitro-PAPS: pyridylazo-N-propyl-N-sulfopropylaminoPhenol method; NO: Nitric oxide; NR: Not reported; OCPC method: o-Cresolphthalein Complexone method; OSI: Oxidative stress index; P: Periodontitis; PD: Probing depth; PI: Plaque index; Protein CO: Protein carbonyl; qPCR: Quantitative Real-Time polymerase chain reaction; SMAC: Small molecule antioxidant capacity; SOD: superoxide dismutase; TAC: Total antioxidant capacity; TOS: Total oxidant status; † data obtained by WebPlotDigitizer online software.

Appendix - Table 2 Quality assessment according to Fowkes and Fulton.

Guideline	Checklist		ALLEN et al. (2011)	LATHA et al. (2018)	PENDYALA et al. (2013)	PUSHPARANI et al. (2013)	PUSHPARANI (2015)	SHETTY et al. (2016)	TAKEDA et al. (2006)	TRIVEDI et al. (2014)	VINCENT et al. (2018)
	Objective:	Common design:									
Study design appropriate to objectives?	Prevalence	Cross sectional	NA	NA	NA	0	0	NA	0	NA	NA
	Prognosis	Cohort	0	NA	NA	NA	NA	NA	NA	NA	NA
	Treatment	Controlled trial	NA	0	NA	NA	NA	0	NA	NA	NA
	Cause	Cohort, case-control	NA	NA	0	NA	NA	NA	NA	0	0
Study sample representative?	Source of sample		+	+	+	+	+	+	+	+	+
	Sampling method		+	+	+	+	+	0	+	+	+
	Sample size		+	++	+	+	+	+	+	+	++
	Entry criteria/exclusions		0	+	0	+	+	+	++	+	+
	Non-respondents		NA	NA	NA	NA	NA	NA	NA	NA	NA
Control group acceptable?	Definition of controls		++	0	0	++	++	++	++	+	++
	Source of controls		+	+	+	+	+	0	+	+	+
	Matching/randomisation		++	++	+	++	++	0	+	+	+
	Comparable characteristics		++	+	+	++	++	++	0	0	+

Quality of measurements and outcomes?	Validity	+	0	0	+	+	0	+	0	+
	Reproducibility	NA	NA	0	0	0	0	0	0	0
	Blindness	NA	NA	NA	NA	NA	+	NA	NA	NA
	Quality control	+	0	0	++	++	0	0	0	0
Completeness?	Compliance	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Drop outs	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Deaths	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Missing data	0	0	0	0	0	0	0	0	0
Distorting influences?	Extraneous treatments	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Contamination	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Changes over time	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Confounding factors	++	0	+	++	++	0	0	+	0
	Distortion reduced by analysis	++	0	++	++	++	0	0	+	0
Summary questions	Bias - Are the results erroneously biased in a certain direction?	YES	YES	YES	YES	YES	YES	YES	YES	YES
	Confounding - Are the any serious Confounding or other distorting influence?	YES	NO	NO	YES	YES	NO	NO	NO	NO
	Chance - Is it likely that the results ocurred by chance?	YES	YES	YES	YES	YES	YES	YES	YES	YES

Appendix - Table 3 Assessment of the certainty of evidence (GRADE)

Certainty assessment							
N° of datasets	**Design of the studies**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Certainty**
MDA							
2	Observational*	Serious[a]	Serious[b]	Not serious	Serious[c]	None	⊕◯◯◯ VERY LOW
TAC							
2	Observational	Serious[a]	Serious[b]	Not serious	Serious[c]	None	⊕◯◯◯ VERY LOW
Magnesium							
2	Observational*	Serious[a]	Not serious	Not serious	Serious[c]	None	⊕◯◯◯ VERY LOW

* Baseline data from intervention studies were considered.
a. The certainty of the evidence was downgraded in one level because the studies had important methodological limitations and it is likely that they may have altered the results.
b. The certainty of the evidence was downgraded by one level because the studies reported different effects.
c. The certainty of the evidence was downgraded by one level due to the reduced number of individuals considered in the synthesis (less than the threshold of 400 recommended by GRADE).

6. MANUSCRITO II

REDOX HOMEOSTASIS BIOMARKERS IN SALIVA AND NUCLEAR CHANGES IN EPITHELIAL CELLS OF INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS AND PERIODONTITIS

Running title: OXIDATIVE STRESS, T2DM AND PERIODONTITIS

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One-Sentence Summary: Redox homeostasis biomarkers levels and nuclear alterations in periodontitis individuals with or without type 2 diabetes mellitus reflect an unbalanced redox balance due to the inflammatory manifestation of diseases present in the OS.

Clinical relevance: Periodontal diseases are highly associated with several inflammation-related systemic diseases, mainly, type 2 diabetes mellitus. Oxidative stress (OS) plays an important role in the pathogenesis of these diseases and hypothesized that OS arising from periodontal lesions may be an essential cause of systemic inflammation. Our data demonstrate that FBS and %HbA1c are associated with worse periodontal conditions. The positive correlations among periodontal parameters, MN and NA (condensed chromatin cells, karyorrhectic cells, and pyknotic cells), and salivary Redox homeostasis biomarkers (GSH and UA) reflect worse periodontal conditions and of the T2DM parameters. So, these nuclear alterations may act as a marker for periodontitis and present a role associated with oxidative stress in the pathogenesis of T2DM with periodontal disease. However, the frequencies of cells with nuclear buds, binucleated and karyolitic, and TAC, total proteins and TBARs concentrations do not seem to play a major role in the pathogenesis of periodontal manifestations in T2DM individuals. Based on the results of this study, these biomarkers indicate the presence of systemic alterations and supporting periodontal tissues and, therefore, their use enables determining the severity of these diseases and a plan for the appropriate treatment for each individual.

Abstract

Aim: To evaluate salivary redox homeostasis biomarkers levels in the periodontitis and type 2 diabetes mellitus, and correlate with periodontal parameters and markers of nuclear alterations in cheek epithelial cells.

Materials and Methods: A total of 60 participants were allocatted into three groups equally: type 2 diabetes with periodontitis (DPE); non-diabetes with periodontitis (PE); healthy without periodontal disease (HC). After periodontal measurements, cheek epithelial cells and saliva samples were collected. DNA damage was determined by counting micronucleus (MN) and nuclear abnormalities (NA) in epithelial cells and oxidative stress levels were determined by reduced glutathione (GSH), uric acid (UA), total antioxidant capacity, thiobarbituric acid reactive substances, and total proteins. Numeric data were tested by Mann-Whitney and Kruskal-Whallis tests, qualitative variables by chi-square test and correlations by the spearman coeficiente, and linear and logistic regression were performed, adopting the significance level of 5%.

Results: MN, karyorrhectic, condensed chromatin, pyknotic cells frequencies, GSH and UA were significantly higher in DPE group followed PE and HC groups ($p<0.05$). Fasting blood glucose, glycated hemoglobin, MN and NA frequencies (karyorrhectic, condensed chromatin and pyknotic cells), reduced glutathione and uric acid showed a positive mild to moderate correlation with periodontitis progression parameters ($p<0.05$). Linear regression analysis showed that categorized GSH had correlation with gingival bleeding ($p = 0.002$) and TBARs ($p = 0.020$) and UA with bleeding on probing ($p = 0.001$) and TAC ($p = 0.001$). Logistic regression analysis showed that categorized GSH levels had correlation with BOP (OR = 1.121 [95% CI, 1.025-1.225]) and SUP (OR = 0.155 [95% CI, 0.029-0.838]).

Conclusions: Positive correlations among periodontal parameters, MN and NA, and salivary redox homeostasis biomarkers (GSH and UA) reflects worse periodontal conditions and of the type 2 diabetes mellitus parameters. Salivary biomarkers were effective for detecting the functioning of the redox balance and nuclear damage in cheek epithelial cells.

Keywords: oxidative stress; DNA damage; periodontitis; type 2 diabetes mellitus.

1. Introduction

Type 2 diabetes mellitus is a chronic inflammatory disease characterized by a relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin that induces an exaggerated proinflammatory state, oxidative stress (OS), and damage to periodontal tissues (Chapple & Mathews, 2007; Manfredi et al., 2004). In the progression of periodontitis, a loss of periodontal attachment occurs due to the interplay between the pathogenic microbiota and an inappropriate host response with high production and release of inflammatory cytokines (Hajishengallis, 2015; Martínez-García & Hernández-Lemus, 2021).

Proinflammatory cascades that cause tissue damage are increased in inflammatory conditions like periodontitis and type 2 diabetes mellitus (T2DM) and this causes an increase of reactive oxygen species (ROS) levels by a higher formation of Advanced Glycation End-products (AGEs). It is considered the link between these diseases and their pathobiology implicates in OS (Takeda et al., 2006).

ROS activity can damage cells by a variety of mechanisms, including peroxidation of lipid membranes, protein inactivation, oxidation of important enzymes and carbohydrates, and induction of DNA damage (Chapple & Mathews, 2007), either by a reduction in antioxidant defense or by increased in the production of these free radicals (Sies, 2015). When the damage occurs in cells genetic material whose function is to transfer genetic information to later generations results in teratogenic or carcinogenic consequences (Eyfjord & Bodvarsdottir, 2005). In the mutations resulting from oxidative DNA lesions, beyond often occur oncogenes and tumor-suppressing genes silenced in cancers, micronucleus (MN) and nuclear abnormalities (NA) are also detected in epithelial cells (Thomas et al., 2009).

The increase in ROS of local and systemic forms found in periodontitis and T2DM can significantly affect the functioning pattern of several tissues and is closely related to the comorbidities associated with T2DM (Gumus et al., 2016; Turrens, 2003). These diseases can be explained by the local and systemic concentrations of some OS biomarkers (Silva et al., 2015).

In this context, the aim of this study was to evaluate redox homeostasis biomarkers levels in saliva of individuals with periodontitis and T2DM, and correlate them with periodontal parameters and markers of nuclear damage and cell death or cytotoxicity in cheek epithelial cells.

2. Materials and methods

2.1 Participants

The present observational study was conducted in the Department of Dental Clinic of the Dental School and in the Division of Nutrology of the Clementino Fraga Filho Hospital of the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, from February 2019 to May 2022. All study participants received an oral hygiene kit, attended a motivational lecture with guidance on oral hygiene (teeth brushing and flossing), were provided dental care according to their needs (after sample collection), and signed the informed consent form (ICF) agreeing to join the study. The study was approved by the Ethics Committee on Human Research from the Clementino Fraga Filho Hospital (n. 3.807.461).

Sixty patients were recruited for the study, distributed equally em three groups: T2DM with periodontitis (DPE), non-diabetes with periodontitis (PE), and healthy without periodontal disease (HC). Sample size calculation was performed using specific software (WinPepi software (http://www.brixtonhealth.com/pepi4windows.html), using the standard-deviation (SD) for catalase (CAT) published previously (Trivedi et al., 2014). It was estimated a required sample of 17 individuals per group with an expected precision of approximately 95% confidence interval (CI) for the difference between means (D) = D -0.070 to D +0.070, with an error of 5% and a power of 80%. Considering a percentage of loss of 20%, a total of 20 individuals per group was adopted in the current study.

Eligible participants were from both sexes, aged 30-70 years, with at least 10 natural teeth. All T2DM participants were diagnosed with T2DM according to the WHO classification and were metabolically controlled using oral hypoglycemic agents and/or insulin. None of the participants within the PE and HC groups were taking any prescription medication. Exclusion criteria included chronic kidney disease, malignant neoplasms, Human Immunodeficiency Virus-Positive (HIV) diagnosis, Acquired Immunodeficiency Syndrome (AIDS), pregnant or lactating women, immunosuppression by medication, autoimmune diseases, evidence of other systemic diseases ASA III and IV, smokers or former-smokers for less than twenty years, users of orthodontic appliances, history of use of steroidal or non-steroidal anti-inflammatory drugs in the three months prior to the study, use of antibiotics or any dietary supplements including antioxidant supplements in the six months prior to the study, following any specific diets

beyond their usual diets during the last six months, as well as individuals who underwent supra and subgingival scaling and/or periodontal surgery in the six months before enrollment.

2.2 Periodontal diagnosis

Complete periodontal clinical measurements were performed at six sites per tooth at all teeth, excluding third molars. The parameters probing depth (PD) and clinical attachment level (CAL) were recorded at the nearest mm using a North Carolina periodontal probe (Hu-Friedy; Chicago, IL, USA). The periodontal examination was performed by calibrated examiner. The intra-class correlation coefficient for CAL and PD were 0.89 and 0.91, respectively. Clinical diagnosis of periodontal status was determined as follows: periodontal health was defined as <10% of sites with bleeding on probing (BOP), no PD or CAL ≥4 mm; and periodontitis was defined as PD ≥4 mm and CAL ≥4 mm in two or more sites of non-adjacent teeth, and BOP >10% (Da Silva-Boghossian et al., 2011; Caton et al., 2018).

2.3 Saliva sampling

Samples were collected in the morning (10:00-12:00 am). Subjects have been instructed not to eat or drink, except mineral water non-carbonated (300 ml) 1H before sampling. Whole saliva samples were collected into Eppendorf tubes without any external stimulation. Salivary samples were centrifuged at 10,000 g for 10 min at 4 °C to remove cell debris. The resulting supernatant was stored at -80 °C until analysis.

2.4 Anthropometric and sorologic measurements

Body mass index (BMI) was calculated based on measures of body weight in kilograms divided by the square of height in meters. The systolic and diastolic blood pressure (in mmHg) was determined as the mean of two measurements. Blood samples were collected after an overnight fast for each subject. Serum was obtained by centrifuging the blood at 1500 rpm for 10 min. Glycated hemoglobin (HbA1c), analyzed by the high-performance liquid chromatography method (Biosystems S.A, Costa Brava, Spain) was expressed in percentage, with a reference value of 5 to 7%. Serum glucose was measured by the electrochemiluminescence method. Serum creatinine levels were measured by the colorimetric method. These analysis were performed in the Clementino Fraga Filho Hospital, Rio de Janeiro, Rio de Janeiro, Brazil.

2.5 Demographic characteristics of the participants

Data about gender, weight, years since diabetes diagnosis, history of COVID-19, vaccination against COVID-19, number of vaccine doses against COVID-19 and the medications used by patients of DPE group were evaluated.

2.6 Biochemical assay

Glutathione (GSH) was assayed in 100 mL of saliva pipetted into Eppendorf tubes containing 200 mL of TCA 10% solution that was mixed and centrifuged at 4000 g for 10 min at 16° C. To 200 mL of the supernatant 700 mL of 400 mM Tris-HCl buffer, pH 8.9, was added followed by the addition of 100 mL of 2.5 mM 5,50-dithiobis (2-nitrobenzoic acid) (DTNB) dissolved in 40 mM Tris–HCl buffer pH 8.9. After 10 min at room temperature, the extinction of the samples was measured at 412 nm in EZ READ 400 microplate Reader. Blank consisted of DTNB instead of sample; its extinction was subtracted from the test sample extinction before matching it with the standard curve (50–1000 mM).

Thiobarbituric acid reactive substances (TBARs) were assayed with 100 mL of saliva mixed with 500 mL TCA 35% plus 500 mL Tris–HCl (200 mM, pH 7.4) and incubated for 10 min at room temperature. One mL of 2 M Na2SO4 and 55 mM thiobarbituric acid solution was added, and the samples were incubated at 95 °C for 45 min. The samples cooled on ice for 5 min, then mixed after the addition of 1 mL TCA 70%. Finally, the samples were centrifuged at 15.000 g for 3 min and the absorbance of the supernatant was read at 530 nm. A molar extinction coefficient was used ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$) and the results were expressed as nmol/mL. Malondialdehyde (MDA) was assayed with a commercial kit following the instructions from the manufacturer (Sigma-AldrichTM, St. Louis, MO).

Uric acid concentration was determined by colorimetric assay (BioclinTM; Minas Gerais, Brazil). Results were expressed in mg/dL.

Total protein concentration was determined using the Biuret method, using BSA as a standard as described by Gornall et al. (1949). The results were expressed in g/dL. Lactate was measured using a commercial kit (BioclinTM; Minas Gerais, Brazil).

TAC was determined through the DPPH radical reduction test (2,2-diphenyl-1-picrylhydrazyl), according to Janaszewsza and Bartosz (2002). 20 µl of sample, 480 µl of 10 mM sodium-potassium phosphate buffer solution (pH 7.4) were added. To 500 µl of

(sample + buffer), 500 µl of 0.1 mM solution (methanol + 2,2-diphenyl-1-picrylhydrazyl) was added. Samples were homogenized and incubated in the dark at room temperature for 30 min. Then centrifuged for 3 min at 700xg for 5 min and absorbance was read at 520nm.

2.7 Cell culture and Buccal Micronucleus Cytome (BMCyt) Assay

Minimally invasive sampling was performed after rinsing the mouth with chlorhexidine 0.12%. Exfoliated cells samples from cheek mucosa were spread directly onto two separate pre-cleaned and pre-coded slides and the cells were extended over the other glass slide. Slides were dried at room temperature, fixed with ethanol p.a. for 2 h, and then stained with acridine orange (CAS no. 10127023; Sigma-Aldrich, St. Louis, MO, USA). Pre-coded slides were examined by one reader, who blindly counted the MNs and NAs, including binucleated cells, cells with nuclear buds, and karyolitic, karyorrhectic, condensed chromatin, and pyknotic cells. The criteria used for scoring MN and NA were according to those described by Thomas et al. (2009), and the number of cells with MN and NAs were evaluated among 500 cells using a Zeiss Axioimager D2 microscope (Carl Zeiss, Oberkochen, Germany) equipped with a fluorescence microscopy mode (objective 630 and 960; Olympus, Tokyo, Japan). The capture of fluorescence imaging was done using a Zeiss filter set 00 (488000-0000-000; excitation: BP 530/585 nm; beam splitter: FT 600 nm; and emission: LP 515 nm).

2.8 Data analysis

Descriptive statistics included medians (interquartile range) and frequency values. Additionally, mean and SD were obtained for MN, NA, laboratory data, and periodontal parameters, as well as percentages of the following variables: laboratory data and categoric clinical parameters. For the assessment of the OS due to the great range of found values, hence median and interquartile ranges were calculated. Kolmogorov-Smirnov normality test to analyze data demonstrated that most variables had not a normal distribution. Significant differences among and between groups were verified using the Kruskal-Wallis and Mann-Whitney tests, respectively. Correlation analysis between variables was calculated by Spearman's correlation coefficient (rho) and by linear regression analysis using the stepwise method and multivariate logistic regression analysis (forward Wald method). Only variables showing significance at $p<0.05$ in the univariate model were included in the multivariate analysis to calculate odds ratio (OR)

and 95% confidence intervals (CI). Significance level was set at 5%. All analysis were performed using the Statistical Program for Social Sciences (SPSS v22.0) for Windows (SPSS, Inc., Chicago, IL, USA).

3. Results

The study population included sixty adults from both sexes (43 females and 17 males), equally distributed among the study groups (Table 1). There were no significant differences between groups for gender, weight, BMI, creatinine, history of COVID-19, vaccination against COVID-19, and number of vaccine doses against COVID-19. Individuals in the HC (33.00 ± 12.13) group were younger compared to DPE (60.50 ± 6.76) and PE (52.50 ± 11.57) groups ($p<0.01$; Kruskal-Wallis test). Mean FBS and %HbA1c were significantly higher in the DPE group (107.00±57.02 and 8.05 ± 1.86, respectively) compared to PE (87.50 ± 6.59 and 5.15 ± 0.53, respectively) and HC (91.50 ± 6.65 and 5.10 ± 0.37) groups, <0.001. Inversely, the number of individuals answering “yes” for alcohol consumption was significantly higher in the HC group compared to other groups ($p=0.003$).

The stage of the periodontitis was more severe in the DPE group compared to the PE group (Table 2). The DPE group presented sighnificantly higher percentage of dental biofilm (DB), gingival bleeding (GB), BOP, suppuration (SUP), dental calculus (DC), %moderate and %deep PD, %moderate and %severe CAL compared to other groups ($p<0.001$). Moreover, the DPE group had significantly higher number of missing teeth ($p<0.001$).

Figure 1 illustrates the different cell types of the study groups. The presence of micronucleus and pyknotic cells was detected with significantly higher frequency in the DPE group compared to PE and HC groups, $p<0.05$ (Table 3). Cells with condensed chromatin were significantly more frequent in the DPE group compared to the HC group, $p<0.05$; while karyorrhectic cells were detected less frequently in the HC group compared to DPE and PE groups, $p<0.001$.

The median values of salivary redox homeostasis biomarkers and the statistical evaluation between different groups are shown in Table 4. The DPE group had significantly higher values of GSH (27.40 ± 5.31), and UA (5.60±1.03) than PE and HC groups ($p<0.05$).

The correlation analysis demonstrated that %HbA1c and years with T2DM had the strongest positive correlation among all tested variables (rho = 0.906, $p<0.001$), as

displayed in Table 5. Other positive strong correlations were found between %HbA1c and periodontal parameters (%BOP, rho = 0.580, p<0.001, %GB, rho = 0.610, p<0.001; %DB, rho = 0.628, p <0.001; %moderate PD, rho = 0.569, p<0.001; %moderate CAL, rho = 0.576, p<0.001; %severe CAL, rho = 0.603, p<0.001; and missing teeth, rho = 0.579, p<0.001).Years with T2DM also showed further strong positive correlations with periodontal parameters, including %BOP (rho = 0.588, p<0.001), %GB (rho = 0.622, p<0.001), %DB (rho = 0.603, p <0.001), %moderate PD (rho = 0.536, p<0.001), %moderate CAL (rho = 0.563, p<0.001), %severe CAL (rho = 0.587, p<0.001), and missing teeth (rho = 0.584, p<0.001). Additionally, FBS had a positive strong correlation with %HbA1c (rho = 0.504, p<0.001).

Nuclear alterations also showed a strong positive correlation with periodontal parameters, including correlation between KAC and %GB (rho = 0.589, p<0.001), %moderate PD (rho = 0.582, p<0.001), %moderate CAL (rho = 0.550, p<0.001), and missing teeth (rho = 0.589, p<0.001). The variable years with T2DM was strongly correlated with PYC (rho = 0.517, p<0.001) and MN (rho = 0.523, p<0.001). A further correlation was found between PYC and %HbA1c (rho = 0.523, p<0.001) (Table 5).

Final model of the linear regression analysis using a stepwise method (Table 6) demonstrated that categorized GSH was associated with GB (p = 0.002) and TBARs (p = 0.020). On the other hand, UA correlated with BOP (p = 0.001) and TAC (p = 0.001). Logistic regression analysis (forward Wald) was used to examine the correlations of categorized GSH and categorized UA with periodontal and T2DM parameters. Of all parameters entered in the model, categorized GSH levels were correlated with BOP (OR = 1.121 [95% CI, 1.025-1.225]) and SUP (OR = 0.155 [95% CI, 0.029-0.838]) (Table 7). Categorized UA levels did not present significant correlations with evaluated parameters.

4. Discussion

OS can produce a wide variety of effects on human health. ROS are presented in elevated levels in chronic diseases, such as T2DM and periodontitis, and it can cause several modifications in the physiological mechanisms taking to DNA damage, lipid peroxidation, and protein denaturation (Chapple & Mathews, 2007). A review of evidence regarding the effect of periodontal treatment in patients with periodontitis and diabetes suggested that inflammation is likely to link the two diseases (Baeza et al., 2020) and that scaling and root planing reduced oxidative stress biomarkers levels taking to an impact on metabolic control and reduction of systemic inflammation (Silva et al., 2018). Saliva

may constitute a first line of defense against free radical-mediated OS, that's because the mastication of food, for example, promotes a variety of such reactions including lipid peroxidation (Pendyala, Thomas, & Joshi, 2013).

However, many studies have been focused on only one or a low number of OS biomarkers, and such a restricted approach only provides limited information (Silva et al., 2018), since there are few studies that correlate them with other methods of detecting these biomarkers. This study evaluated salivary parameters of OS, antioxidant status in saliva, and nuclear alterations in cheek epithelial cells from 60 individuals with periodontitis and T2DM or systemically healthy compared to periodontally and systemically healthy individuals. We observed differences in periodontal clinical parameters, %HbA1c and FBS among groups, confirming that the DPE group had significantly greater severity of periodontitis, as described by other studies (Nelson et al., 1990; Morita et al., 2012; Myllymäki et al., 2018).

Ethanol metabolism, from the fermentation or distillation of vegetables, fruits, and grains, is directly involved in the production of reactive oxygen species (ROS) that results in decreased antioxidant activity and in the depletion of GSH levels (Das & Vasudevan, 2007). Our data showed differences significantly to etilism among groups with consumption of alcohol in greater amounts by the HC group, but had no correlation with GSH levels and they had lower OS levels.

Individuals with T2DM (Ojeda et al., 2017) and periodontitis (Rao et al., 2020) have presented an increased risk of DNA damage, due to the accumulation of ROS. In this study, the mean frequency of MN, condensed chromatin cells, karyorrhectic cells, and pyknotic cells were significantly higher in the DPE group, slightly lower in the PE group, and lower in the HC group ($p<0.001$). These data corroborate other studies indicating that chronic inflammatory conditions are associated with an increased OS because the increased phagocytic action of neutrophils occurs DNA damage in cells (Corbi et al., 2014; Zamora-Perez et al., 2014; Rao et al., 2020). These markers showed a statistically significant positive mild to moderate correlation with %HbA1c, FBS mean values, and years since diabetes diagnosis that indicate oxidative damage levels in the expression of defense genes and in the DNA of cheek epithelial cells caused by the systemic inflammatory condition of hyperglycemia ($p<0.01$). This suggests an association between metabolic status and an increase in MN number in the buccal mucosa, as shown in the study of Ojeda et al. (2017). Cells with nuclear bud, binucleated

cells, and karyolytic cells had no significant differences between groups nor significant correlation with study variables.

In the current study, MN and some NA (condensed chromatin cells, karyorrhectic cells, and pyknotic cells) presented a correlation clinical with signs of periodontitis: BOP, GB, SUP, DB, PD, CAL, and more specifically with % moderate PD, % moderate CAL and % severe CAL, and also with DC ($p<0.01$). Zamora-Perez et al. (2014) identified an association between periodontal status and an increase in MN and NA mean numbers in buccal mucosa cells. The results showed that periodontitis could be a modifiable risk factor for DNA damage, as periodontitis can be prevented and treated.

The occurrence of periodontitis in individuals with T2DM commonly suggests that both conditions have common elements of pathogenesis as these diseases have inflammation as a result of permanently increased OS levels (Kim et al., 2011). Arana et al. (2017) related that GSH is a reliable biomarker of cellular oxidative stress and T2DM individuals with poor metabolic control have greater degradation of this biomarker and, therefore, significantly lower levels when compared to well-controlled diabetic, pre-diabetic and healthy individuals. In the same line of reasoning, Thomas et al. (2021) found significantly lower levels of GSH in periodontitis individuals with diabetes when compared to periodontitis patients without diabetes and healthy individuals. These studies showed that chronic hyperglycemia and oxidative stress in diabetic patients are accompanied by increased GSH oxidation with the consequent reduction of its levels. However, contrary to these studies, our data showed GSH had higher levels in individuals with more inflammation (DPE group) when compared to non-diabetes with periodontitis and healthy without periodontal disease individuals ($p<0.05$). The occurrence of moderate oxidative stress is a consequence of the increase in enzymatic antioxidant defenses, but the production of great number of free radicals can cause cell damage (Anderson, 1996).

UA is a strong antioxidant in a hydrophilic environment and its levels increase during OS (Sautin & Johnson, 2008). However, due to the scarcity of studies that report salivary UA concentration in T2DM with periodontitis individuals, the comparison of results becomes rather more complicated. In this study, UA had significantly higher levels in the group with T2DM and periodontitis when compared to other study groups. Mussavira et al. (2015) analyzed salivary UA in 53 patients with T2DM and 40 healthy subjects, and an increase in UA concentration was observed in the saliva of patients with diabetes when compared to healthy subjects, according to our findings. Soukup et al. (2012) evaluated in 78 patients with metabolic syndrome, salivary UA and the results

suggest that this biomarker may be useful for monitoring of cardiometabolic risk, corroborating our findings. These data are not in agreement with the study by Miricescu et al. (2013) that analyzed 20 patients with periodontitis and 20 controls. They reported that UA levels were significantly decreased in patients with periodontitis versus controls. Byun et al. (2020) evaluated the association between hyperuricemia (excessive uric acid in the blood) and periodontitis in 8809 with hyperuricemia and 126.465 controls (non-hyperuricemia). Their data showed that the adjusted Odds Ratio of hyperuricemia for periodontitis was lower in all patients than that in the control group. The current study demonstrated that hyperuricemia was associated with periodontitis, and these findings suggest that elevated uric acid levels might have a positive effect on periodontitis.

Linear regression analysis demonstrated significant associations between categorized GSH with GB and TBARs ($p = 0.020$). Logistic regression showed correlation of categorized GSH with BOP and SUP. This showed that GSH is an important biomarker for detectation of inflammation caused by periodontitis, corroborating Kim et al. (2011). UA correlated positively with BOP ($p = 0.001$) and inversely with TAC ($p = 0.001$), confirming its role as an important oxidative stress biomarker. GSH homeostasis can be explained as a result of the oxidant/antioxidant balance, unlike what happens with UA, which is a final product of purine metabolism and is the result of the breakdown of amino acids present in our body's proteins. The increase in salivary GSH may be related to greater production/transport to meet greater antioxidant demand via pentose-phosphate, which results in greater concentration of UA (Mussavira et al., 2015).

According to Chapple et al. (2002), inadequate total antioxidant defense enhances tissue injury due to excessive free radical production in individuals with periodontal disease. A huge diversity of antioxidants is present in a biological system, and estimating TAC reduces the cost and time necessary to measure individual antioxidant species (Baltacıoğlu et al., 2014). A case-control study, comprising 120 male subjects, identified the lowest TAC levels in patients with diabetes and periodontitis, whereas the highest values were present in healthy subjects without periodontitis. This study had no correlated TAC levels between patients with diabetes with and without periodontitis (Pendyala, Thomas, & Joshi, 2013). This agrees with our findings in the DPE group, which had lower median values of TAC, followed in ascending order by PE and HC groups, but no statistically significant difference was found. That TAC levels did not change, perhaps

because the concentrations of the components were not sufficient to generate differences in the assay.

TBARs and total proteins levels were higher, but not significantly, in our group of individuals with T2DM and periodontitis and in the group of individuals with only periodontitis compared to the healthy control group. These comparability results between groups corroborate other studies that showed statistically significant differences in a by-product of lipid peroxidation, such as TBARs, and protein total concentration in saliva of individuals with T2DM with periodontitis, periodontitis without type 2 diabetes and systemically healthy without the periodontal disease (Trivedi et al., 2014; Kejriwal, Bhandary, Thomas, Kumari, 2014; Latha, Uppoor, Nayak, Naik, 2018).

DPE group had higher severity of periodontitis compared to PE group and, in this same group, GSH and UA had significantly higher levels. The increased levels of these biomarkers and nuclear abnormalities along with parameters of elevated periodontal inflammation support the hypothesis of the role of OS in the pathogenesis of periodontitis in T2DM individuals. Our findings showed significant correlations between those parameters and corroborate several studies that report this hypothesis (Takeda et al., 2006; Pendyala, Thomas, & Joshi, 2013; Trivedi et al., 2014; Zamora-Perez et al., 2014; Arana et al., 2017; Latha et al., 2018; Vincent et al., 2018).

Our study presents some limitations that need to be acknowledged. Firstly, is a lack of a group with T2DM without periodontitis, which would enable us to conclude whether the levels of salivary antioxidants are definitely related to the diabetic status, independently of the clinical periodontal situation. Secondly, the lack of investigation of those studied biomarkers in the serum and in the gingival crevicular fluid to compare with the biomarkers in saliva, and with nuclear alterations in cheek epithelial cells. That would be interesting to establish whether saliva might be an efficient fluid in the investigation of the OS biomarkers. Finally, a heterogeneous profile among groups regarding age and use of drugs for metabolic changes control, such as the statins, which reduce the NAD+/NADH ratio and OS by blocking the generation of ROS (Lim & Barter, 2014). However, it is worth mentioning that, despite that, OS levels were higher in the studied patients with T2DM.

In conclusion, FBS and %HbA1c are associated with worse periodontal conditions, with %HbA1c showing stronger correlations with worse periodontitis scores. These diseases present the action of oxidizing agent levels elevated and alterations in the function of antioxidants, resulting in greater cellular damage if they remain in this

inflammatory condition. The positive correlations among periodontal parameters, MN and NA (condensed chromatin cells, karyorrhectic cells, and pyknotic cells), and salivary redox homeostasis biomarkers (GSH and UA) reflect worse periodontal conditions and of the T2DM parameters. Therefore, it may act as a marker for periodontitis and present a role associated with OS in the pathogenesis of T2DM with periodontal disease. However, the frequencies of cells with nuclear buds, binucleated and karyolitic, and total proteins and TBARs concentrations do not seem to play a major role in the pathogenesis of periodontal manifestations of T2DM.

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Conflict of Interest

The authors report no conflicts of interest.

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Table 1. Demographic and clinical characteristics of the study groups

Variables	T2DM with periodontitis - DPE (n = 20)	Periodontitis - PE (n = 20)	Healthy controls – HC (n = 20)	p-value
Gender (male/female)	4/16	6/14	7/13	0.563 0.568†
Age (years)	60.50 ± 6.76	52.50 ± 11.57	33.00 ± 12.13	<0.01$^{*/***}$ <0.001$^{**/\dagger}$
Weight (kg)	78.65 ± 17.71	70.00 ± 11.05	72.50 ± 13.90	NS 0.502†
BMI (kg/m^2)	29.75 ± 6.11	25.45 ± 3.32	25.05 ± 4.82	NS 0.016†
FBS (mg/dl)	107.00±57.02	87.50 ± 6.59	91.50 ± 6.65	<0.001$^{*/**/\dagger}$
%HbA1c (%)	8.05 ± 1.86	5.15 ± 0.53	5.10 ± 0.37	<0.001$^{*/**/\dagger}$
Creatinine (mg/dl)	0.80 ± 0.16	0.90 ± 0.13	0.90 ± 0.11	NS 0.112†
Years since diabetes diagnosis	13.00 ± 7.13	n.a.	n.a.	n.a.
Etilist (yes/no)	4/16	3/17	13/7	0.003$^{*/**/***}$ <0.001†
COVID-19 (yes/no)	11/9	8/12	10/10	0.627 0.632†
Vaccinated against COVID-19 (yes/no)	20/0	19/1	20/0	0.362 0.368†
Vaccine doses against COVID-19	3.00 ± 0.36	3.00 ± 1.13	3.00 ± 0.58	NS 0.366†
Medication				
Oral hypoglycaemics	0.90 ± 0.30	n.a.	n.a.	n.a.
Regular insulin	0.35 ± 0.49	n.a.	n.a.	n.a.
NPH insulin	0.70 ± 0.47	n.a.	n.a.	n.a.
Statins	0.90 ± 0.31	n.a.	n.a.	n.a.
Ace inhibitors	0.25 ± 0.44	n.a.	n.a.	n.a.
β-blockers	0.55 ± 0.51	n.a.	n.a.	n.a.
Channel blockers	0.30 ± 0.47	n.a.	n.a.	n.a.
ARB	0.40 ± 0.50	n.a.	n.a.	n.a.

Diuretic	0.55 ± 0.51	n.a.	n.a.	n.a.
Cellular phosphodiesterase inhibitor	0.05 ± 0.22	n.a.	n.a.	n.a.
Anticoagulant	0.20 ± 0.41	n.a.	n.a.	n.a.
Gastric acid suppressant	0.15 ± 0.36	n.a.	n.a.	n.a.
Antiemetic	0.05 ± 0.22	n.a.	n.a.	n.a.
Hypolipidemic	0.10 ± 0.30	n.a.	n.a.	n.a.

Note: BMI, Body mass index; FBS, Fasting blood sugar; %HbA1c, Glycosylated hemoglobina percentual; NPH insulin Neutral Protamine Hagedorn insulin; Ace inhibitors, Angiotensin-converting enzyme inhibitors; ARB, Angiotensin II receptor blockers; NS, not significant, using Mann-Whitney's U test or chi-square test (χ^2); n.a., not applicable. Medication data were presented as mean ± SD and other data were presented as median ± SD (interquartile range). Mann-Whitney's U test was used for numeric variables, and chi-square test (χ^2) was used for nominal variables. *indicates statistically significant differences between DPE and PE groups. **indicates statistically significant differences between DPE and HC groups. ***indicates statistically significant differences between PE and HC groups. †indicates p value, using Kruskal-Wallis test.

Table 2. Periodontal data of the study groups (mean ± SD)

Parameters	T2DM with periodontitis - DPE (n = 20)	Periodontitis - PE (n = 20)	Healthy controls – HC (n = 20)	p-value
DB (%)	80.57 ± 15.02	49.33 ± 32.92	14.71 ± 7.75	<0.01$^{*/***}$ <0.001$^{**/\dagger}$
BOP (%)	68.03 ± 22.00	47.76 ± 24.20	7.11 ± 4.98	<0.01* <0.001$^{**/***/\dagger}$
GB (%)	72.12 ± 20.59	49.05 ± 23.36	9.04 ± 5.13	<0.01* <0.001$^{**/***/\dagger}$
DC (%)	50.24 ± 22.29	32.05 ± 20.85	7.30 ± 5.00	<0.05* <0.001$^{**/***/\dagger}$
PD (mm)	3.05 ± 1.19	2.70 ± 0.75	2.11 ± 0.14	<0.01$^{**/***}$ 0.003†
CAL (mm)	3.86 ± 1.18	2.84 ± 0.80	2.14 ± 0.14	<0.05* <0.01$^{**/***/\dagger}$
SUP (%)	0.78 ± 1.38	0.05 ± 0.20	0,00 ± 0.00	<0.05* <0.01** 0.006†
% shallow PD (0-3mm)	65.60 ± 28.15	80.30 ± 18.70	99.27 ± 1.04	<0.05* <0.001$^{**/***/\dagger}$
% moderate PD (4-6 mm)	30.20 ± 22.16	18.40 ± 16.84	0.73 ± 1.04	<0.05* <0.001$^{**/***/\dagger}$
% deep PD (≥7 mm)	4.30 ± 8.52	1.45 ± 2.21	0.00 ± 0.00	<0.01$^{**/***}$ 0.008†
% slight CAL (0-2 mm)	54.50 ± 28.56	77.54 ± 18.84	99.00 ± 1.38	<0.01* <0.001$^{**/***/\dagger}$
% moderate CAL (3-4 mm)	30.00 ± 14.78	19.99 ± 16.46	1.00 ± 1.38	<0.05* <0.001$^{**/***/\dagger}$
% severe CAL (≥5 mm)	15.50 ± 21.32	2.46 ± 3.46	0.00 ± 0.00	<0.01* <0.001$^{**/***/\dagger}$
Missing teeth	14.50 ± 5.83	9.05 ± 5.75	0.95 ± 1.87	<0.01* <0.001$^{**/***/\dagger}$

Note: DB, dental biofilm; BOP, bleeding on probing; GB, gingival bleeding; DC, dental calculus; PD, probing depth; CAL, clinical attachment loss; SUP, supuration; NS, not significant; n.a., not applicable. Mann-Whitney U test was used to analyse the differences between groups. *indicates statistically significant differences between DPE and PE groups. **indicates statistically significant differences between DPE and HC groups. ***indicates statistically significant differences between PE and HC groups. †indicates statistically significant differences among groups, using Kruskal-Wallis test.

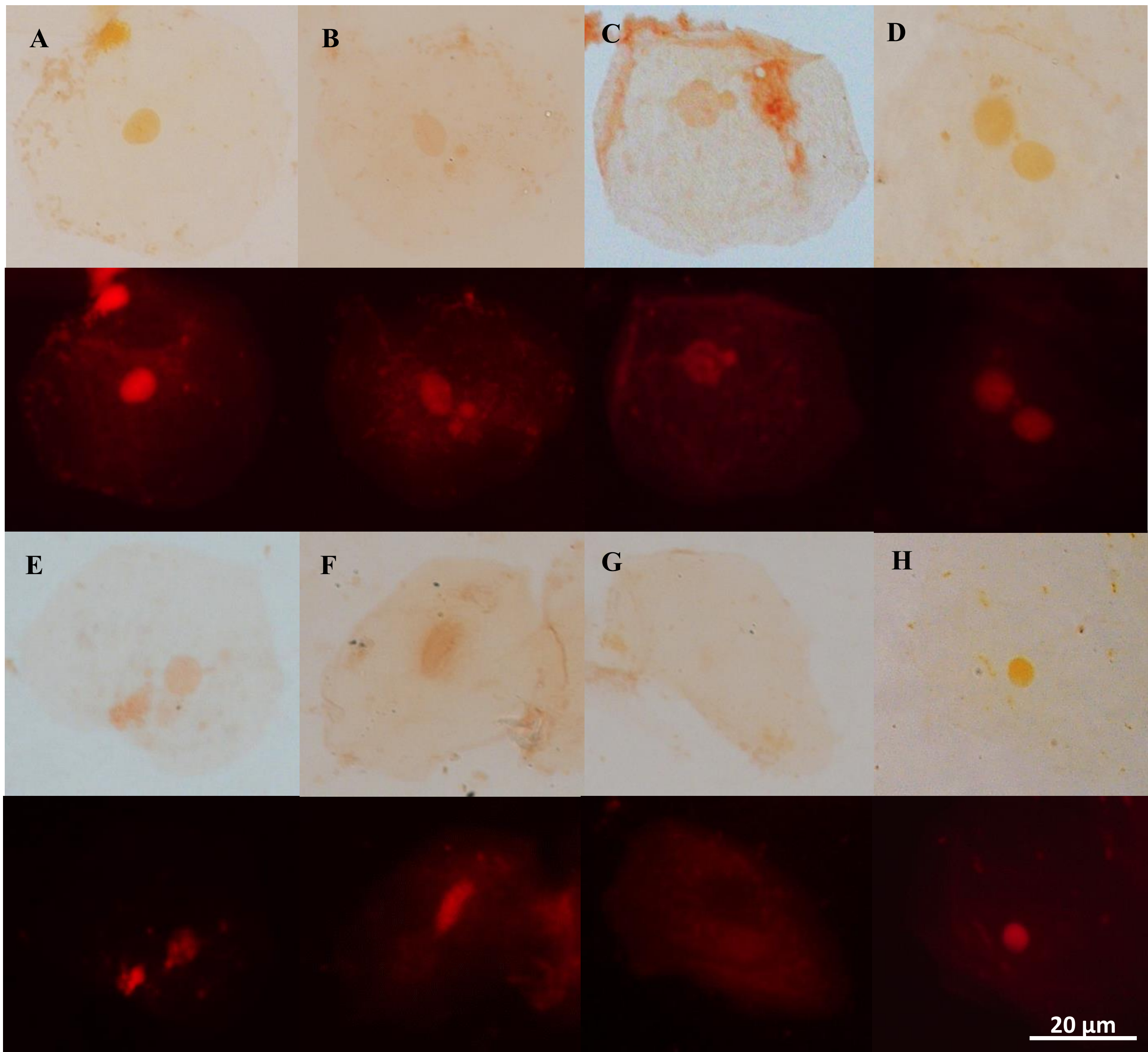

Figure 1. Images of the different cell types stained using acridine orange scored in the BMCyt assay viewed by transmitted light or under fluorescence with a Zeiss filter set 00 – 5 Rox (Rhodamine) (Zeiss Axioimager D2 microscope - Carl Zeiss, Oberkochen, Germany). Differentiated cell (A), MN (B), cell with nuclear bud (C), binucleated cell (D), karyorrhectic cell (E), condensed chromatin cell (F), karyolytic cell (G) and pyknotic cell (H)

Table 3. Frequency of micronuclei and other nuclear abnormalities in cheek epithelial cells (mean ± SD) in individual with periodontitis and T2DM

Nuclear abnormalities	Groups			p-value
	T2DM with periodontitis - DPE - (n = 20)	**Periodontitis - PE - (n = 20)**	**Healthy controls – HC - (n = 20)**	
Micronuclei	5.70 ± 4.02	1.95 ± 2.01	1.90 ± 3.53	<0.001$^{*/**/\dagger}$
Cells with nuclear buds	1.05 ± 1.35	0.50 ± 1.05	0.55 ± 0.75	NS
Binucleated cells	0.35 ± 0.81	0.30 ± 0.66	0.20 ± 0.41	NS
Condensed chromatin cells	1.10 ± 1.25	0.60 ± 0.82	0.25 ± 0.55	<0.05** 0.035†
Karyorrhectic cells	2.00 ± 1.77	1.25 ± 1.11	0.10 ± 0.31	<0.001$^{**/***/\dagger}$
Karyolitic cells	0.40 ± 0.68	0.35 ± 0.49	0.15 ± 0.37	NS
Pyknotic cells	2.30 ± 1.75	1.00 ± 0.97	0.15 ± 0.49	<0.05* <0.001$^{**/\dagger}$ 0.002***

Note: Mann-Whitney's U test was used to analyse the differences between groups: *DPE vs. PE, **DPE vs. HC and ***PE vs. HC. †indicates statistically significant differences among groups, using Kruskal-Wallis test. NS, not significant.

Table 4. Oxidative stress biomarkers salivary in the study groups (median ± SD)

Oxidative stress biomarkers	Groups			p-value
	T2DM with periodontitis (DPE)	Periodontitis (PE)	Healthy controls (HC)	
GSH (mg/dl)	27.40 ± 5.31	26.65 ± 4.90	23.90 ± 4.98	0.01**/*** 0.018†
TAC (µmol/dl)	33.15 ± 2.98	33.20 ± 3.95	33.35 ± 4.43	NS
TBARs (nmol/ml)	19.00 ± 7.27	22.30 ± 21.88	18.00 ± 25.37	NS
Total proteins (mg/dl)	2.20 ± 2.01	2.30 ± 0.13	2.30 ± 0.28	NS
UA (mg/dl)	5.60 ± 3.53	5.30 ± 0.99	5.25 ± 0.81	0.01** 0.043†

Note: GSH, reduced glutathione; TAC, total antioxidant capacity; TBARs, thiobarbituric acid reactive substances; UA, uric acid; Mann-Whitney's U test was used to analyse the differences between groups: *DPE vs. PE, **DPE vs. HC and ***PE vs. HC. †indicates statistically significant differences among groups, using Kruskal-Wallis test. *indicates statistically significant differences among groups. NS, not significant.

Table 5. Main correlations among oxidative stress markers, nuclear alterations, periodontal clinical parameters and T2DM data

	%HbA1c	FBS	Years T2DM	MN	CCC	KAC	PYC	GSH	UA
	Spearman correlation coefficient (p-value)								
%BOP	**0.580 (<0.001)**	0.299 (0.020)	**0.588 (<0.001)**	0.446 (<0.001)	0.370 (0.004)	0.398 (<0.001)	0.452 (<0.001)	0.317 (0.014)	0.312 (0.015)
%GB	**0.610 (<0.001)**	0.255 (0.049)	**0.622 (<0.001)**	0.408 (<0.001)	0.333 (0.009)	**0.589 (<0.001)**	0.439 (<0.001)	0.366 (0.004)	0.318 (0.013)
%DB	**0.628 (<0.001)**	0.295 (0.022)	**0.603 (<0.001)**	0.443 (<0.001)	0.307 (0.017)	0.457 (<0.001)	0.415 (0.001)	0.351 (0.006)	0.378 (0.003)
%Mod PD	**0.569 (<0.001)**	0.326 (0.011)	**0.536 (<0.001)**	0.470 (<0.001)	0.345 (0.007)	**0.582 (<0.001)**	0.408 (0.001)	0.365 (0.004)	0.302 (0.019)
%Deep PD	NS	NS	NS	0.285 (0.027)	NS	NS	NS	NS	NS
%Mod . CAL	**0.576 (<0.001)**	0.373 (0.003)	**0.563 (<0.001)**	0.429 (0.001)	0.412 (0.001)	**0.550 (<0.001)**	0.479 (<0.001)	0.307 (0.017)	0.349 (0.006)
%Sev CAL	**0.603 (<0.001)**	0.279 (0.031)	**0.587 (<0.001)**	0.406 (0.001)	0.257 (0.048)	0.448 (<0.001)	0.286 (0.027)	0.263 (0.042)	0.266 (0.040)
Missing teeth	**0.579 (<0.001)**	0.358 (0.005)	**0.584 (<0.001)**	0.325 (0.011)	0.391 (0.002)	**0.589 (<0.001)**	0.343 (0.007)	0.305 (0.018)	0.314 (0.015)
%HbA1c	-	**0.504 (<0.001)**	**0.906 (<0.001)**	0.477 (<0.001)	0.366 (<0.01)	0.317 (<0.01)	**0.523 (<0.001)**	NS	NS
FBS	-	-	0.487 (<0.001)	NS	0.385 (0.002)	0.316 (0.014)	0.388 (0.002)	NS	NS
Years T2DM	-	-	-	**0.523 (<0.001)**	0.326 (0.011)	0.314 (0.015)	**0.517 (<0.001)**	NS	0.320 (0.013)
MN	-	-	-	-	NS	NS	0.293 (0.023)	0.258 (0.046)	NS
CCC	-	-	-	-	-	NS	0.338 (0.008)	NS	NS
KAC	-	-	-	-		-	NS	0.387 (0.002)	NS
PYC	-	-	-	-	-	-	-	NS	0.261 (0.044)
GSH	-	-	-	-	-	-	-	-	0.265 (0.041)

Note: BOP, bleeding on probing; GB, gingival bleeding; DB, dental biofilm; Mod. PD, moderate probing depth; deep PD, deep probing depth; Mod. CAL, moderate clinical atachment level; Sev. CAL, severe clinical atachment level; HbA1c, glycated hemoglobin; FBS, fasting blood sugar; MN, micronucleus; CCC, condensed chromatin cells; KAC, karyorrhectic cells; PYC, pyknotic cells; GSH, reduced glutathione; UA, uric acid; NS, non-signficant. The strongest correlations are highlighted in bold.

Table 6. Linear regression of the association between redox homeostasis biomarkers and variables with statistically significant differences among study groups

Predictor variables in the final model	Dependent variables	
	Categorized GSH[†]	Categorized UA[‡]
Analysis 1		
GB (%)	0.351 (0.006)[§]	NI
TAC (µmol/dl)	NI	-0.370 (0.004)[§]
Adjusted R^2	0.108	0.122
Analysis 2		
GB (%)	0.399 (0.002)[§]	NI
TAC (µmol/dl)	NI	-0.402 (0.001)[§]
TBARs (nmol/ml)	0.286 (0.020)[§]	NI
BOP (%)	NI	0.401 (0.001)[§]
Adjusted R^2	0.175	0.273

Note: BOP, bleeding on probing; GB, gingival bleeding; GSH, reduced glutathione; TAC, total antioxidant capacity; TBARs, thiobarbituric acid reactive substances; UA, uric acid; NI: not included in the final model for the referred dependent variable. [†]Categorization: concentration of GSH 25 mg/dl (50% of the cases) thru highest = 1 and 0 mg/dl thru 24.9 mg/dl = 0. [‡]Categorization: concentration of UA 5.4 mg/dl (53.3% of the cases) thru highest = 1 and 0 mg/dl thru 24.9 mg/dl = 0. [§]Standardized β coefficient (p-value). Excluded variables on the analysis: age, etilism, glycosylated hemoglobin percentual, fasting blood sugar, dental biofilm, dental calculus, probing depth (shalow, moderate and deep percentuals), clinical attachment loss (slight, moderate and severe percentuals), supuration and total proteins.

Table 7. Logistic regression analysis of GSH and variables with statistically significant differences among study groups

Categorized GHS†							
						95% CI to OR	
Variables	β	SE	Wald	p	OR	Lower	Upper
Constant	-730.781	2073.452	0.124	0.725	<0.001		
BOP (%)	0.114	0.046	6.214	0.013	1.121	1.025	1.225
SUP (%)	-1.865	0.861	4.689	0.030	0.155	0.029	0.838

Note: BOP, bleeding on probing; GSH, reduced glutathione; SUP, supuration. †Categorization: concentration of GSH 25 mg/dl (50% of the cases) thru highest = 1 and 0 mg/dl thru 24.9 mg/dl = 0. Excluded variables on the analysis: age, etilism, glycosylated hemoglobin percentual, fasting blood sugar, dental biofilm, gingival bleeding, dental calculus, probing depth (shalow, moderate and deep percentuals) and clinical attachment loss (slight, moderate and severe percentuals).

7. DISCUSSÃO

Durante o normal cumprimento das funções biológicas celulares fundamentais, ocorre a geração de radicais livres que atuam como mediadores para a transferência de elétrons nas várias reações bioquímicas. Sua síntese, em adequadas proporções, como consequência da cadeia transportadora de elétrons, ativação de genes e participação de mecanismos de defesa durante o processo de infecção é responsável pela geração de ATP (energia) (LOBO *et al.*, 2010). Porém, quando há um desequilíbrio persistente nas reações de oxidação-redução celular, pendendo para as ROS, seja por uma redução na defesa antioxidante ou pelo aumento na produção ou na atividade delas, ocorre OS (SIES, 2015).

As cascatas pró-inflamatórias que causam dano tecidual são aumentadas em condições inflamatórias, como periodontite e DM2 (SHETTY et al., 2016). Ambas as doenças estão associadas a hiperinflamação e aumento do OS. E por isso, a literatura indica uma inter-relação bidirecional entre DM2 e periodontite, sendo o DM2 responsável por aumentar o risco de periodontite e a periodontite por ter um efeito negativo no controle glicêmico em indivíduos suscetíveis (GROSSI e GENCO, 1998; MEALEY, 2006, TAYLOR *et al*., 2013).

O aumento do dano oxidativo (SEZER *et al*., 2012; SILVA *et al*., 2015) e níveis mais baixos de antioxidantes (BALTACIOĞLU *et al*., 2014; D'AIUTO *et al*., 2010) são encontrados em pacientes com ambas as doenças em comparação aos saudáveis e em pacientes com apenas periodontite em comparação a indivíduos sistemicamente e periodontalmente saudáveis. A condição inflamatória periodontal pode ser explicada pelas concentrações locais e sistêmicas de alguns biomarcadores do OS (TAKEDA *et al.*, 2006; PUSHPA RANI *et al.*, 2013; TRIVEDI *et al.*, 2014; PUSHPA RANI, 2015; SILVA *et al*., 2015; SHETTY *et al.*, 2016; LATHA *et al*. 2018; VINCENT *et al.*, 2018). Este estudo avaliou parâmetros salivares de estresse oxidativo, status antioxidante na saliva e alterações nucleares em células epiteliais da mucosa jugal em 60 indivíduos com periodontite e DM2, e com periodontite e sistemicamente saudáveis em comparação a indivíduos periodontalmente e sistemicamente saudáveis. Observou-se diferenças nos parâmetros clínicos periodontais entre os grupos, onde o grupo DPE apresentou gravidade significativamente maior da periodontite, conforme descrito por muitos estudos (NELSON *et al*., 1990; MORITA *et al*., 2012; MYLLYMÄKI *et al*., 2018).

Patógenos periodontais ativam a resposta do hospedeiro levando à liberação de neutrófilos e macrófagos, que são as fontes primárias de radicais livres na periodontite.

Conforme evidenciado por inúmeros estudos, (KINANE, 2001; CHAPPLE e MATHEWS, 2007; GUMUS *et al.*, 2016; ARANA *et* al., 2017; VINCENT *et al.*, 2018; THOMAS *et al.*, 2021) é bem conhecido que a periodontite induz um baixo grau de inflamação, criando assim um processo oxidativo ambiente com reduzida capacidade antioxidante. Os dados do presente estudo corroboram os encontrados na literatura em relação aos níveis da TAC, que foram maiores nos pacientes sistemicamente e periodontalmente saudáveis (HC), seguido pelos pacientes com periodontite e sem alterações sistêmicas (PE) e pelos pacientes com DM2 e periodontite (DPE).

Porém, no presente estudo, detectou-se para outros dois biomarcadores antioxidantes (GSH e UA), maiores concentrações estatisticamente significantes ($p<0,05$) em indivíduos com diabetes tipo 2 e periodontite em comparação aos indivíduos com periodontite sem alterações sistêmicas e indivíduos sistemicamente e periodontalmente saudáveis. Acredita-se que o presente dado atribuiu-se ao fato de serem pacientes em constante processo de quadros inflamatórios com oscilações de piora da saúde, devido às comorbidades atreladas ao DM2 e em decorrência da presença da periodontite. Anderson (1996) relata que a ocorrência de estresse oxidativo moderado é proveniente do aumento da ação das substâncias antioxidantes enzimáticas e da ação da resposta do hospedeiro, propriamente dita. Ainda assim, GSH e UA mostraram ter correlação, ainda que fraca, com os parâmetros de progressão da periodontite (SS, ISG, IB, CA, percentual de PS moderada e percentual de NCI moderado) ($p<0.05$) e GSH teve correlação positiva com dentes perdidos e células cariorréticas ($p<0.01$), mostrando que esses biomarcadores salivares foram capazes de identificar a inflamação e os danos celulares presentes no DM2 e na periodontite.

UA é um forte antioxidante em um ambiente hidrofílico e seus níveis aumentam durante a OS (Sautin & Johnson, 2008). No entanto, devido à escassez de estudos que relatam a concentração deste biomarcador em saliva nos indivíduos com DM2 e periodontite, a comparação dos resultados torna-se um pouco mais complicada. Neste estudo, o UA apresentou níveis significativamente mais altos em indivíduos com DM2 e periodontite quando comparados aos demais grupos de estudo. Mussavira *et al*. (2015) analisaram UA salivar em 53 pacientes com DM2 e 40 indivíduos saudáveis, e foi observado um aumento na concentração desse biomarcador na saliva de pacientes com DM quando comparados a indivíduos saudáveis, corroborando com nossos achados. Esses dados não estão de acordo com o estudo de Miricescu *et al*. (2013) que analisaram 20 pacientes com periodontite e 20 controles. Eles relataram que os níveis de UA

diminuíram significativamente em pacientes com periodontite quando comparados aos controles. Byun *et al*. (2020) avaliaram a associação entre hiperuricemia (excesso de ácido úrico no sangue) e periodontite em 8809 com hiperuricemia e 126.465 controles (não hiperuricemia). Os dados mostraram que o aOR (OR ajustado) de hiperuricemia para periodontite foi menor em todos os pacientes do que no grupo controle. Este estudo demonstrou que a hiperuricemia estava associada à periodontite, e esses achados preliminares sugerem que níveis elevados de ácido úrico podem ter um efeito positivo na periodontite. No presente estudo UA mostrou uma correlação fracamente inversa com TAC (rho=-0.414, p=0.001) sugerindo que o UA pode ser um biomarcador mais sensível para o processo inflamatório na periodontite e quando associada à DM2, ele é sintetizado em maior quantidade na tentativa de controlar a quantidade de ROS presentes.

A análise de regressão linear demonstrou associações significativas entre GSH categorizado com ISG (p = 0,002) e TBARs (p = 0,020). A regressão logística mostrou correlação do GSH categorizado com SS e SUP. Isso mostrou que o GSH é um importante biomarcador para a detecção da inflamação causada pela periodontite, corroborando Kim *et al*. (2011). O UA correlacionou-se positivamente com SS (p = 0,001) e inversamente com TAC (p = 0,001), confirmando seu papel como um importante biomarcador do balanço redox. A homeostase da GSH pode ser explicada como resultado do equilíbrio oxidante/antioxidante, ao contrário do que acontece com o UA, que é um produto final do metabolismo das purinas e é o resultado da quebra de aminoácidos presentes nas proteínas do nosso corpo. O aumento do GSH salivar pode estar relacionado à maior produção/transporte para atender a maior demanda antioxidante via pentose-fosfato, o que resulta em maior concentração de UA (Mussavira et al., 2015).

TBARs é um fator evidenciado durante a peroxidação lipídica (LPO) como um mecanismo potencial subjacente à doença periodontal associada ao diabetes (HOOSHMAND *et al*., 2005). Entre os vários produtos finais de baixo peso molecular da peroxidação lipídica, o malondialdeído (MDA) é a TBARs mais frequentemente medida como um índice de LPO e é encontrado em elevadas concentrações em indivíduos com periodontite e diabetes, podendo ser utilizado para avaliar o dano oxidativo nesses pacientes (HOOSHMAND *et al*., 2005; TRIVEDI *et al.*, 2014; LATHA *et al.*, 2018). No presente estudo, mesmo que sem diferenças estatísticas, os indivíduos inflamados, seja pela presença do DM2 com periodontite ou somente da periodontite, apresentaram maiores concentrações de TBARs em comparação aos indivíduos sistemicamente e periodontalmente saudáveis.

O desequilíbrio do SNA (sistema nervoso autonômico) caracteriza a patogênese da disfunção metabólica que ocorre no DM2. Na fase inicial ocorre a hiperinsulinemia compensatória, pela ativação do sistema nervoso parassimpático (SNP) e/ou diminuição da atividade do sistema nervoso simpático (SNS), em decorrência da resistência insulínica das células. Somente após à falência das células beta do pâncreas é que ocorre o quadro de hiperglicemia, com a função simpática constantemente estimulada (WU *et al.,* 2015; GALICIA-GARCIA *et al.,* 2020). Esse desequilíbrio do SNA presente no DM2 pode estar associado à diminuição do fluxo salivar relatado por esses pacientes e ao aumento da quantidade de proteínas, compostos orgânicos e inorgânicos na saliva, já que, segundo Bortollini et al. (2009), as glândulas salivares são inervadas por esse sistema e a estimulação da função parassimpática causa vasodilatação, enquanto que da simpática gera vasoconstricção. Nossos dados em relação à concentração de proteínas totais, mostraram concentrações semelhantes entre os grupos de estudo sem diferenças estatisticamente significantes, talvez porque não houve excitação suficiente das células inflamatórias e das ROS para uma grande estimulação simpática nos grupos DM2P e PE.

Devido ao acúmulo de ROS, os indivíduos com DM2 (OJEDA *et al*., 2017) e periodontite (RAO *et al*., 2020) têm apresentado um risco aumentado de dano ao DNA, Neste estudo, a frequência média de MN, células de cromatina condensada, células cariorreticas e células picnóticas foi significativamente maior no grupo DPE, ligeiramente menor no grupo PE e menor no grupo HC ($p>0,001$). Esses dados corroboram outros estudos que indicam que condições inflamatórias crônicas estão associadas ao aumento do OS, pois ação fagocítica dos neutrófilos aumentada é capaz de gerar danos ao DNA celular (CORBI *et al*., 2014; ZAMORA-PEREZ *et al*., 2014; RAO *et al*., 2020). MN mostrou uma correlação positiva leve a moderada estatisticamente significativa com %HbA1c (rho=0.477) e duração do diabetes em anos (rho=0.523), já as células cariorréticas, as com cromatina condensada e as picnóticas com %HbA1c (rho=0.336, rho=0.336, rho=0.523), valores médios de FBS (rho=0.385, rho=0.316, rho=0.388) e duração do diabetes em anos (rho=0.326, rho=0.314, rho=0.517), respectivamente, o que indicam níveis de dano oxidativo na expressão de genes de defesa e no DNA de células epiteliais da bochecha causadas pela condição inflamatória sistêmica de hiperglicemia ($p<0,01$). Isso sugere uma associação entre o estado metabólico e o aumento do número de MN na mucosa bucal, conforme demonstrado no estudo de Ojeda *et al.* (2017). Células com broto nuclear, células binucleadas e células cariolíticas não apresentaram diferenças significativas entre os grupos e nem correlação siginificativa com as variáveis do estudo.

Nossos dados mostraram que todos os parâmetros periodontais apresentaram correlação mútua moderada a forte (rho>0,5) e correlação positiva leve (rho entre 0,3 e 0,5) entre os parâmetros de progressão da periodontite com MN, células de cromatina condensada, células cariorréticas e células picnóticas. Todas essas correlações foram estatisticamente significativas. No presente estudo, MN e algumas NA (células de cromatina condensada, células cariorréticas e células picnóticas) apresentaram correlação e sinais de progressão da periodontite: sangramento à sondagem (SS), índice de sangramento gengival (ISG), índice de cálculo dentário (CA), Índice de supuração (SUP), além das medidas de profundidade de sondagem (PS) e o nível clínico de inserção (NCI) e maiores correlações com percentual de PS moderada, percentual de NCI moderado e percentual de NCI avançado (p<0,01). Nossos achados corroboram o estudo de Zamora-Perez et al. (2014) em 160 pacientes com periodontite avançada, que identificaram uma associação entre o estado periodontal e o aumento dos números médios de MN e NA nas células da mucosa bucal. Os resultados mostraram que a periodontite pode ser um fator de risco modificável para danos ao DNA, pois a doença pode ser prevenida e tratada. Além disto, os dados mostram mais uma vez o impacto sistêmico da periodontite, que não pode ser mais ser vista como uma doença localizada.

Em relação às limitações do presente estudo, podemos citar, primeiramente, a ausência do grupo com indivíduos com DM2 sem periodontite, o que nos permitiria concluir se os níveis de antioxidantes salivares estão definitivamente relacionados com o estado diabético, independentemente da situação clínica periodontal, porém no local onde o estudo foi realizado não tiveram pacientes com saúde periodontal. Segundo, a falta de biomarcadores séricos e do fluido gengival para comparar com a saliva e com alterações nucleares nas células epiteliais da bochecha, a fim de enriquecimento do estudo e saber se a saliva é um bom fluido para estudar os biomarcadores do estresse oxidativo. Por fim, um perfil heterogêneo entre os grupos quanto à idade e o uso de medicamentos para controle de alterações metabólicas, como a estatina, que reduz o estresse oxidativo ao bloquear a geração de ROS, por reduzir a relação NAD+/NADH (Lim & Barter, 2014). O que não pareceu interferir nos nossos resultados, já que os níveis de OS foram maiores em pacientes com DM2.

O percentual de HbA1c mostrou uma forte correlação com anos desde o diagnóstico de diabetes (r=0,906, p<0,001) e uma correlação positiva moderada com dentes perdidos, IB, SS, ISG, CA, percentual de PS moderado, percentual de NCI moderado e % CAL grave (p<0,001). Os valores médios de FBS mostraram uma

correlação positiva moderada com %HbA1c (r=0,504) (p<0,001), uma correlação positiva leve com anos desde o diagnóstico de diabetes, dentes perdidos, % PD moderada, CAL, % CAL moderada, células cariorréticas, células de cromatina condensada e células picnóticas (p<0,01), e uma correlação negativa leve com percentual de PS leve e percentual de NCI leve (p<0,01). Anos desde o diagnóstico de diabetes mostrou uma correlação positiva moderada com dentes perdidos (r=0,584), todos os parâmetros periodontais de progressão da doença, uma correlação positiva leve com células cariorréticas e células de cromatina condensada (p<0,01). Esses dados sugerem que FBS e %HbA1c elevados estão associados à progressão da periodontite, conforme os estudos de Nelson *et al.* (1990), Morita *et al.* (2012) e Myllymäki *et al.* (2018), sendo %HbA1c mostrando correlações mais fortes com piores escores de periodontite e que o tempo de duração do DM2 é um importante fator na progressão da periodontite e do surgimento de alterações nucleares nas células da mucosa jugal.

O desenvolvimento do presente estudo apresentou grandes dificuldades para sua realização em virtude do surgimento da pandemia da COVID-19. Muitos pacientes interromperam o tratamento presencial nos serviços da nutrologia do hospital universitário Clementino Fraga Filho e outros, acometidos pela doença, faleceram. Os laboratórios para realizar o processamento das salivas fecharam as portas e quando retornaram suas atividades foi de forma restrita e já estavam comprometidos com outras pesquisas mais antigas. Começou então a busca pelo local onde processá-las, no qual encontramos o laboratório de fisiopatologia do exercício da UERJ. Outra dificuldade foi encontrar um laboratório para leitura das lâminas em fluorescência e captura das imagens, o que só tornou-se possível com a disponibilidade de infra-estrutura na Unimicro (CCS-UFRJ). Porém, mesmo com todos os percalços foi possível desenvolvermos uma pesquisa observacional de caráter inédito na área da medicina periodontal e que visou estreitar a relação entre a periodontite e DM2.

8. CONCLUSÃO

Os estudos incluídos na revisão sistemática apresentaram falhas metodológicas, poucos marcadores estudados e ausência de homogeneidade na avaliação dos marcadores do balanço redox, bem como, a baixíssima certeza de evidência entre os estudos incluídos. Assim, não foi possível determinar se há ou não diferenças nos níveis de estresse oxidativo em indivíduos com DM2 associado à periodontite.

O presente estudo transversal mostrou correlações positivas entre os parâmetros periodontais, MN, NA (cromatina condensada, células cariorréticas e picnóticas) e os biomarcadores salivares do estresse oxidativo (GSH e UA), sendo esses, os que refletem piores condições periodontais e dos parâmetros do DM2.

TAC, TBARs, proteínas totais, frequências de células com broto nuclear, células binucleadas e células cariolíticas não apresentaram correlações siginificativas com as variáveis do estudo e nem diferenças entre os grupos, o que se mostraram invalidados como biomarcadores do estresse oxidativo.

Fica claro que alguns dos biomarcadores na saliva (GSH e UA) foram eficazes para detecção do desequilíbrio do balanço redox e dos danos nucleares em células epiteliais da mucosa jugal.

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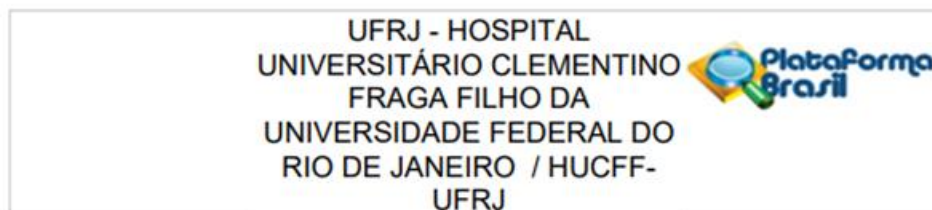
ANEXO I

PARECER DE APROVAÇÃO NO COMITÊ DE ÉTICA EM PESQUISA


UFRJ - HOSPITAL UNIVERSITÁRIO CLEMENTINO FRAGA FILHO DA UNIVERSIDADE FEDERAL DO RIO DE JANEIRO / HUCFF-UFRJ

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: EFEITO DO TRATAMENTO PERIODONTAL NÃO-CIRÚRGICO SOBRE BIOMARCADORES DO ESTRESSE OXIDATIVO DO DIABETES MELLITUS TIPO 2 EM INDIVÍDUOS COM PERIODONTITE

Pesquisador: WALDER JANSEN

Área Temática:

Versão: 2

CAAE: 25047119.1.0000.5257

Instituição Proponente: UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

Patrocinador Principal: Capes Coordenação Aperf Pessoal Nivel Superior
UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

DADOS DO PARECER

Número do Parecer: 3.807.461

Apresentação do Projeto:

Protocolo 458-19. Respostas recebidas em 31.12.2019.

As informações colocadas nos campos denominados "Apresentação do Projeto", "Objetivo da Pesquisa" e "Avaliação dos Riscos e Benefícios" foram retiradas do documento intitulado "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1429008.pdf" (postado na Plataforma Brasil em 31/12/2019).

INTRODUÇÃO

3 INTRODUÇÃO3.1 Associação entre periodontite e diabetes mellitus tipo 2A Doença Periodontal (DP) compreende um grupo de doenças inflamatórias de origem infecciosa, tais como Gengivites e Periodontites, que se caracterizam clinicamente pela destruição dos tecidos de proteção e sustentação do dente, podendo evoluir até a perda do elemento dentário, sendo resultado da interação entre bactérias presentes no biofilme dental subgengival e o sistema imune do hospedeiro (LINDHE et al., 2008). A relação entre a periodontite e as doenças sistêmicas tem sido motivo de estudos ao redor do mundo há pelo menos 3 décadas e mostram que a periodontite

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Folha de Rosto	folha_de_rosto_assinada.pdf	19:47:25	WALDER JANSEN	Aceito
Orçamento	orcamento.pdf	31/10/2019 19:18:29	WALDER JANSEN	Aceito
Declaração de Pesquisadores	declaracao_de_pesquisadores_com_assinatura.pdf	31/10/2019 19:03:24	WALDER JANSEN	Aceito
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Cronograma	Cronograma_Projeto.pdf	31/10/2019 18:23:50	WALDER JANSEN	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

RIO DE JANEIRO, 23 de Janeiro de 2020

Assinado por:
Carlos Alberto Guimarães
(Coordenador(a))

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ANEXO II

PUBLICAÇÃO CIENTÍFICA DURANTE O DOUTORAMENTO

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ANEXO III

COMPROVANTE DE SUBMISSÃO DO ARTIGO I

Journal of Dental Research

Journal of
Dental Research

OXIDATIVE STRESS IN TYPE 2 DIABETES AND PERIODONTITIS: A SYSTEMATIC REVIEW

Journal:	*Journal of Dental Research*
Manuscript ID	JDR-22-1230
Manuscript Type:	Clinical Review
Date Submitted by the Author:	10-Dec-2022
Complete List of Authors:	Lobão, Walder; UFRJ, Menezes, Claudia; UFRJ Marañón-Vásquez, Guido; Federal University of Rio de Janeiro Health Sciences Centre, Masterson, Daniele; Universidade Federal do Rio de Janeiro, Central Library of the Health Science Center, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil Maia, Lucianne; Federal University of Rio de Janeiro, Pediatric Dentistry and Orthodontics Barros, Maria Cynésia; UFRJ Silva-Boghossian, Carina; UFRJ Sansone, Carmelo; UFRJ
Keywords:	Diabetes, Periodontal Medicine, Oxidative stress, Systematic reviews and evidence-based medicine, Inflammation
Abstract:	Oxidative stress is negatively influenced by type 2 diabetes mellitus and when associated with periodontitis the damage is greater. The authors' aim in this systematic review was to verify the scientific evidence for difference of oxidative stress biomarkers in individuals with type 2 diabetes mellitus with and without periodontitis. Were included observational studies, baseline data of prospective and interventional studies, searched through the PubMed, Scopus, Embase, Web of Science, Cochrane Library, Virtual Health Library databases, and other sources. Risk of bias and quality assessment were evaluated from Fowkes and Fulton guidelines and GRADE. Of 689 relevant articles, the authors included 9 studies for the final analysis. Among those studies, 4 cross-sectional, 3 case-control, and 2 interventional studies were included. The studies reported high concentrations of oxidizing agents and low antioxidants levels in individuals with type 2 diabetes mellitus and periodontitis when compared to with no periodontitis. Due to the high heterogeneity, methodological flaws, and low certainty of the evidence among included studies, it was not possible to determine whether there are or not differences in the oxidative stress levels in individuals with T2DM associated with periodontitis, and therefore, further prospective observational and interventional studies are recommended.

ANEXO IV

FICHA DE ANAMNESE

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
FACULDADE DE ODONTOLOGIA

MÊS/ANO _____/_______

DADOS PESSOAIS:

NOME:____GÊNERO______COR______________

IDADE:_________ DATA DE NASCIMENTO: ______/______/_______ ESTADO CIVIL:__________

PROFISSÃO: ___________________________ ENDEREÇO:__________________________________

____TELEFONES:________________/________________

HISTÓRIA MÉDICA:

É portador de: DM - HbA1c ______GL _____ Tempo com DM _________ Doenças cardiovasculares ( )

Osteoporose ( ) Doença renal crônica ( ) Creatinina ________ Ácido úrico_______ Diálise? Sim( ) Não( )

Peso_______ Alt______ IMC _______ Hipertensão ( ) P.A.________ Epilepsia ( ) Dislipidemia ( )

Outras alterações sistêmicas: _________________ Uso de medicamentos? ____________________________

Teve COVID? Sim ( ) Não ( ) Vacinado/Quantas doses? _________ Quando teve COVID tinha tomado quantas doses?____ Sintomas: Leve( ) moderado/internado( ) Grave/intubado( ) Qual vacina?__________

Está grávida? Sim ( ) Não ( ) Meses?_________________________ Está amamentando? Sim ( ) Não ( )

Fumante? Sim ( ) Não ( ) Há quanto tempo? ______ Quantid/dia:______Carga tabágica (anos-maço) ____

Ex-fumante? Sim ( ) Não ( ) Há quanto tempo deixou de fumar? _______________________________

Período em que fumou _______ Quantos cigarros fumava/dia _______ Etilista ( ) Frequência ___________

HISTÓRIA DENTAL:

É portador de doença gengival? Sim ( ) Não ( ) Há quanto tempo?_______________

Já se submeteu a tratamento gengival? Sim ( ) Não ( ) Que tipo?_______________ Quando?___________

Gengiva sangra com facilidade? Sim ( ) Não ( ) Sente seus dentes com mobilidade? Sim ( ) Não ( )

CONTROLE DE BIOFILME:

Quantas vezes escova seus dentes por dia? _____ Usa fio/fita dental ? Sim ( ) Não ( ) Frequência:______

Faz uso de bochechos? Sim ( ) Não ( ) Qual substância? ______________________________

Outros meios auxiliares de higiene bucal? Sim ( ) Não ( ) Qual?_______________________________

TERMO DE CONSENTIMENTO:

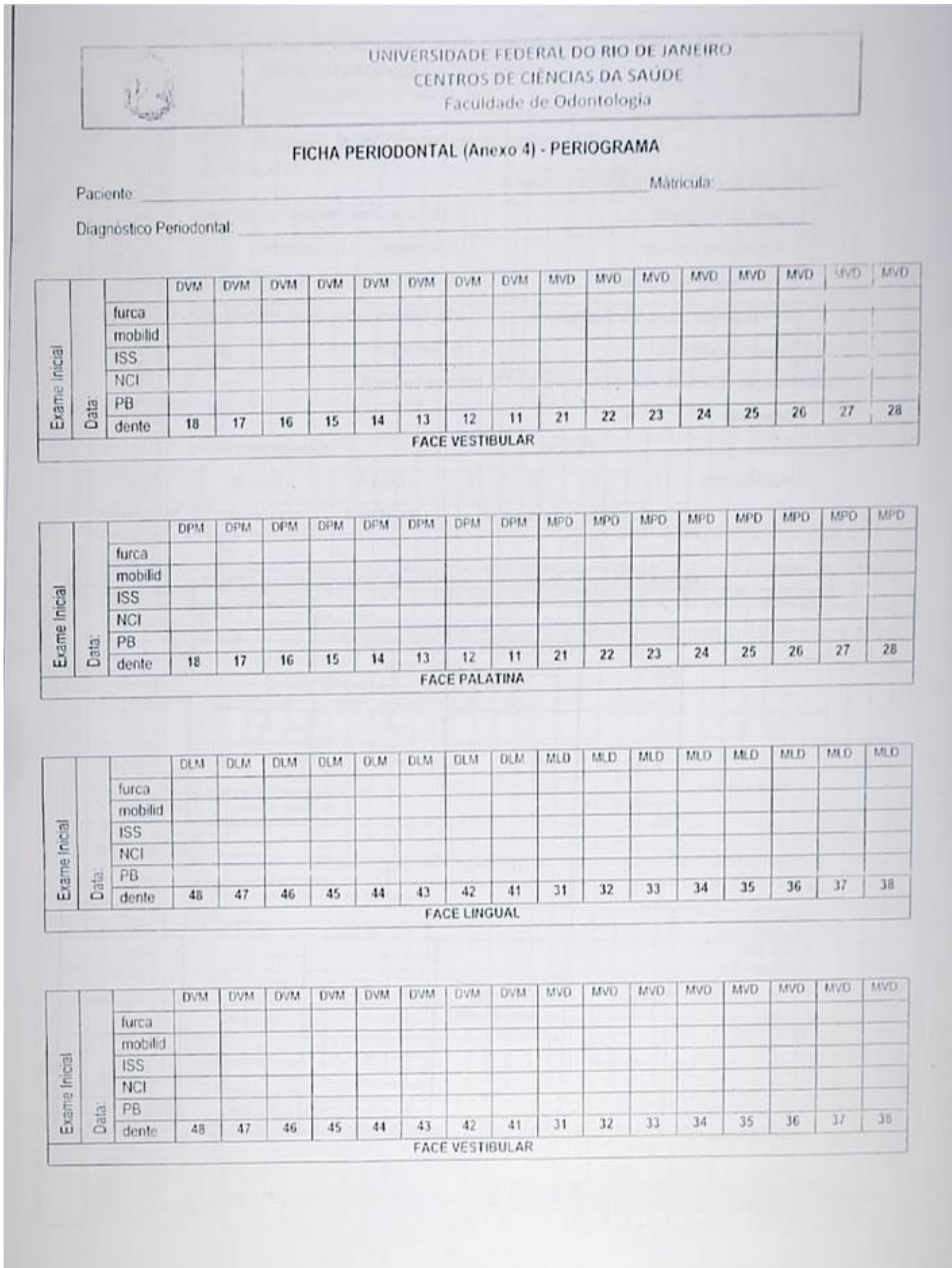
Eu,...CPF.................. .... declaro que todas as informações sobre meu estado de saúde são verdadeiras e que nada omiti no questionário realizado.

Rio de Janeiro, ... de .... de ....

....
Assinatura do Paciente ou Responsável

ANEXO V

PERIOGRAMA



UNIVERSIDADE FEDERAL DO RIO DE JANEIRO
CENTROS DE CIÊNCIAS DA SAÚDE
Faculdade de Odontologia

FICHA PERIODONTAL (Anexo 4) - PERIOGRAMA

Paciente: ______________________ Mátricula: __________

Diagnóstico Periodontal: ______________________

Exame Inicial	Data:		DVM	DVM	DVM	DVM	DVM	DVM	DVM	DVM	MVD	MVD	MVD	MVD	MVD	MVD	MVD	MVD
		furca																
		mobilid																
		ISS																
		NCI																
		PB																
		dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

FACE VESTIBULAR

Exame Inicial	Data:		DPM	DPM	DPM	DPM	DPM	DPM	DPM	DPM	MPD	MPD	MPD	MPD	MPD	MPD	MPD	MPD
		furca																
		mobilid																
		ISS																
		NCI																
		PB																
		dente	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28

FACE PALATINA

Exame Inicial	Data:		DLM	DLM	DLM	DLM	DLM	DLM	DLM	DLM	MLD	MLD	MLD	MLD	MLD	MLD	MLD	MLD
		furca																
		mobilid																
		ISS																
		NCI																
		PB																
		dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

FACE LINGUAL

Exame Inicial	Data:		DVM	DVM	DVM	DVM	DVM	DVM	DVM	DVM	MVD	MVD	MVD	MVD	MVD	MVD	MVD	MVD
		furca																
		mobilid																
		ISS																
		NCI																
		PB																
		dente	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

FACE VESTIBULAR

UNIVERSIDADE FEDERAL DO RIO DE JANEIRO

CENTROS DE CIÊNCIAS DA SAÚDE- FACULDADE DE ODONTOLOGIA- PERIODONTIA

NOME DO PACIENTE: DATA DO EXAME:

ÍNDICES: placa visível (IPV): presença=1 ausência =0 dente ausente X | Índice de sangramento gengival: presença=1 ausência =0 dente ausente X

FATORES RETENTIVOS DE PLACA: cálculo= presença=1 ausência =0 dente ausente X | Restauração deficiente e resto radicular: presença=1 ausência =0 dente ausente X

MAXILA

REGIÃO		ÍNDICES		FATORES RETENTIVOS DE PLACA			REGIÃO		ÍNDICES		FATORES RETENTIVOS DE PLACA		
dente	superfície	IPV	ISG	cálculo	rest.def./cavidade	resto rad.	dente	superfície	IPV	ISG	cálculo	rest.def./cavidade	resto rad.
18	D						21/61	M					
18	V						21/61	V					
18	M						21/61	D					
18	P						21/61	P					
17	D						22/62	M					
17	V						22/62	V					
17	M						22/62	D					
17	P						22/62	P					
16	D						23/63	M					
16	V						23/63	V					
16	M						23/63	D					
16	P						23/63	P					
15/55	D						24/64	M					
15/55	V						24/64	V					
15/55	M						24/64	D					
15/55	P						24/64	P					
14/54	D						25/65	M					
14/54	V						25/65	V					
14/54	M						25/65	D					
14/54	P						25/65	P					
13/53	D						26	M					
13/53	V						26	V					
13/53	M						26	D					
13/53	P						26	P					
12/52	D						27	M					
12/52	V						27	V					
12/52	M						27	D					
12/52	P						27	P					
11/51	D						28	M					
11/51	V						28	V					
11/51	M						28	D					
11/51	P						28	P					

ANEXO VI

Author Guidelines

Journal of Clinical Periodontology now offers Free Format submission for a simplified and streamlined submission process. Read more here.

Sections

1. Submission
2. Aims and Scope
3. Manuscript Categories and Requirements
4. Preparing the Submission
5. Editorial Policies and Ethical Considerations
6. Author Licensing
7. Publication Process After Acceptance
8. Post Publication
9. Editorial Office Contact Details

1. SUBMISSION

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Preprint policy

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For help with submissions, please contact: cpeedoffice@wiley.com

2. AIMS AND SCOPE

The aim of the *Journal of Clinical Periodontology* is to provide a platform for the exchange of scientific and clinical progress in the field of periodontology and allied disciplines, and to do so at the highest possible level. The Journal also aims to facilitate the application of new scientific knowledge to the daily practice of the concerned disciplines and addresses both practicing clinicians and members of the academic community.

The Journal is the official publication of the European Federation of Periodontology but serves an international audience by publishing contributions of high scientific merit in the fields of periodontology and implant dentistry. The journal accepts a broad spectrum of original work characterized as clinical or preclinical, basic or translational, as well as authoritative reviews, and proceedings of important scientific workshops. The journal's scope encompasses the physiology and pathology of the periodontal and peri-implant tissues, the biology and the modulation of periodontal and peri-implant tissue healing and regeneration, the diagnosis, etiology, epidemiology, prevention and therapy of periodontal and peri-implant diseases and conditions, the association of periodontal infection/inflammation and general health, and the clinical aspects of comprehensive rehabilitation of the periodontitis-affected patient.

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

Journal of Clinical Periodontology publishes original research articles, reviews, clinical innovation reports and case reports. The latter will be published only if they provide new fundamental knowledge and if they use language understandable to the clinician. It is expected that any manuscript submitted represents unpublished original research.

i. Original Research Articles

Original Research 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 articles will be published under the heading of clinical periodontology, implant dentistry or pre-clinical sciences and must conform to the highest international standards in the field.

Word limit: 3,500 words maximum, excluding references.
Abstract: 200 words maximum; must be structured, under the sub-headings: Aim(s), Materials and methods, Results, Conclusion(s).
Figures/Tables: Total of no more than 7 figures and tables.

Introduction: should be focused, outlining the historical or logical origins of the study and not summarize the results; exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation.

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. As a condition of publication, authors are required to make materials and methods used freely

available to academic researchers for their own use. This includes antibodies and the constructs used to make transgenic animals, although not the animals themselves.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations.

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 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.

The discussion may usefully be structured with the following points in mind (modified from the proposal by **Richard Horton (2002), The Hidden Research Paper, The Journal of the American Medical Association, 287, 2775-2778)**. Not all points will apply to all studies and its use is optional, but we believe it will improve the discussion section to keep these points in mind.

Summary of key finding

- Primary outcome measure(s)
- Secondary outcome measure(s)
- Results as they relate to a prior hypothesis

Strengths and Limitations of the Study

- Study Question
- Study Design
- Data Collection
- Analysis
- Interpretation
- Possible effects of bias on outcomes

Interpretation and Implications in the Context of the Totality of Evidence

- Is there a systematic review to refer to?
- If not, could one be reasonably done here and now?
- What this study adds to the available evidence
- Effects on patient care and health policy
- Possible mechanisms

Controversies Raised by This Study Future Research Directions

- For this particular research collaboration
- Underlying mechanisms
- Clinical research

ii. Clinical Innovation Reports

Clinical Innovation Reports are suited to describe significant improvements in clinical practice such as the report of a novel surgical technique, a breakthrough in technology or practical approaches to recognized clinical challenges. They should conform to the highest scientific and clinical practice standards.

Word limit: 3,000 words maximum, excluding references.
Main text: should be organized with Introduction; Clinical Innovation Report; Discussion and Conclusion.
Figures/Tables: Total of no more than 12 figures and tables.

iii. Case Reports

Case Reports illustrating unusual and clinically relevant observations are acceptable, but their merit needs to provide high priority for publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered.

Main text: should be organised with Introduction; Case report; Discussion and Conclusion.

iv. Reviews and Systematic Reviews

The Journal primarily publishes invited reviews or systematic reviews by experts in the field.

Unsolicited systematic reviews may be considered under the following conditions:

1. In the submission letter, the authors convincingly articulate the novelty of the findings, and the potential impact of the review on clinical practice, policy or research.
2. There is enough new evidence generated by high quality/large sample size studies that has the potential to modify the conclusions supported by systematic reviews published to date.
3. If not a Cochrane review, the systematic review has been prospectively registered in PROSPERO (https://www.crd.york.ac.uk/prospero/).

Word limit: 4,000 words maximum, excluding references.
Main text: should be organized with Introduction; Review; Discussion and Conclusion.

Revisions and Resubmissions

Please note that all revisions and resubmissions of papers should also include a separate rebuttal and a tracked changes document to assist in peer review.

4. PREPARING THE SUBMISSION

Free Format submission

Journal of Clinical Periodontology now offers Free Format submission for a simplified and streamlined submission process.

Before you submit, you will need:

- Your manuscript: this can be a single file including text, figures, and tables, or separate files – whichever you prefer. All required sections should be contained in your manuscript, including a title page with all author details, including affiliations and email addresses, a statement of clinical relevance, abstract, introduction, methods, results, and conclusions. Figures and tables should have legends. References may be submitted in any style or format, as long as it is consistent throughout the manuscript. If the manuscript, figures or tables are difficult for you to read, they will also be difficult for the editors and reviewers. If your manuscript is difficult to read, the editorial office may send it back to you for revision.
  (Why is this important? We need to make sure your manuscript is suitable for review.
- Statements relating to our ethics and integrity policies:
  - Conflict of interest disclosure
  - Statement of funding source
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  - Patient consent statement (if appropriate)
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- A separate Conflict of Interest form for each author.
  (Why is this important? We need to uphold rigorous ethical standards for the research we consider for publication.)
- Your co-author details, including affiliation and email address. *(Why is this important? We need to keep all co-authors informed of the outcome of the peer review process.)*
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To submit, login at **https://mc.manuscriptcentral.com/jcpe** and create a new submission. Follow the submission steps as required and submit the manuscript.

If you are invited to revise your manuscript after peer review, the journal will also request the revised manuscript to be formatted according to journal requirements as described below.

Cover Letters

A cover letter is mandatory and must be signed by the corresponding author. It is required to confirm that the submitted work is (i) original, (ii) not currently under consideration for publication elsewhere, and (iii) in compliance with all rules stipulated by the Journal.

Parts of the Manuscript

Manuscripts can be uploaded either as a single document (containing the main text, tables and figures), or with figures and tables provided as separate files. Should your manuscript reach revision stage, figures and tables must be provided as separate files. The main manuscript file can be submitted in Microsoft Word (.doc or .docx) format.

Main Text File

Your main document file should include:

i. A short informative title containing the major key words. The title should not contain abbreviations;
ii. The full names of the authors with institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted;
iii. Acknowledgments;
iv. Abstract structured (intro/methods/results/conclusion) or unstructured;
v. Up to seven keywords;
vi. Main body: formatted as introduction, materials & methods, results, discussion, conclusion
vii. References;
viii. Tables (each table complete with title and footnotes);
ix. Figures: Figure legends must be added beneath each individual image during upload AND as a complete list in the text;
x. Appendices (if relevant)

Figures and supporting information should be supplied as separate files.

Authorship

Please refer to the journal's authorship policy the **Editorial Policies and Ethical Considerations section** for details on eligibility for author listing.

Acknowledgments

Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

Conflict of Interest Statement

Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the section 'Conflict of Interest' in the **Editorial Policies and Ethical Considerations section** below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Abstract

The abstract is limited to 200 words in length and should not contain abbreviations or references. The abstract should be organized according to the content of the paper.

For Original Research Articles the abstract should be organized with aim, materials and methods, results and conclusions.

For clinical trials, it is encouraged that the abstract finish with the clinical trial registration number on a free public database such as clinicaltrials.gov.

Keywords

Please provide 1-5 keywords. When appropriate keywords are available, they should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser list at **www.nlm.nih.gov/mesh**. Authors may add specific keywords.

Main Text

All manuscripts should emphasize clarity and brevity. Authors should pay special attention to the presentation of their findings so that they may be communicated clearly. Technical jargon should be avoided as much as possible and be clearly explained where its use is unavoidable.

Clinical Relevance

This section is aimed at giving clinicians a reading light to put the present research in perspective. It should be no more than 100 words and should not be a repetition of the abstract. It should provide a clear and concise explanation of the rationale for the study, of what was known before and of how the present results advance knowledge of this field. If appropriate, it may also contain suggestions for clinical practice.

It should be structured with the following headings: Scientific rationale for study; Principal findings; Practical implications.

Authors should pay particular attention to this text as it will be published in a highlighted box within their manuscript; ideally, reading this section should leave clinicians wishing to learn more about the topic and encourage them to read the full article.

References

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.

References should be prepared according to the Publication Manual of the American Psychological Association (6th edition). This means in text citations should follow the author-date method whereby the author's last name and the year of publication for the source should appear in the text, for example, (Jones, 1998). The complete reference list should appear alphabetically by name at the end of the paper.

A sample of the most common entries in reference lists appears below. Please note that a DOI should be provided for all references where available. For more information about APA referencing style, please refer to the **APA FAQ**. Please note that for journal articles, issue numbers are not included unless each issue in the volume begins with page one.

Journal article

Beers, S. R. , & De Bellis, M. D. (2002). Neuropsychological function in children with maltreatment-related posttraumatic stress disorder. The American Journal of Psychiatry, 159, 483–486. doi:**10.1176/appi.ajp.159.3.483**

Book

Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

Chapter in an Edited Book

Borstrøm, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), Dyslexia: Biology, cognition and intervention (pp. 235–253). London: Whurr.

Internet Document

Norton, R. (2006, November 4). How to train a cat to operate a light switch [Video file]. Retrieved from ***http://www.youtube.com/watch?v=Vja83KLQXZs***

Please note that all unpublished papers (submitted or in press) included in the reference list should be provided in a digital version at submission. The unpublished paper should be uploaded as a supplementary file for review.

Tables

Tables should be self-contained and complement, not duplicate, information contained in the text. They should be supplied as editable files, not pasted as images. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure Legends

Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

Figures

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted.

Click here for the basic figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

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Appendices will be published after the references. For submission they should be supplied as separate files but referred to in the text.

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Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc.

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Note: if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

General Style Points

The following points provide general advice on formatting and style.

- **Abbreviations, Symbols and Nomenclature:** *Journal of Clinical Periodontology* adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. Abbreviations should be kept to a minimum, particularly those that are not standard. Non-standard abbreviations must be used three or more times and written out completely in the text when first used.

Resource Identification Initiative

The journal supports the **Resource Identification Initiative**, which aims to promote research resource identification, discovery, and reuse. This initiative, led by the **Neuroscience Information Framework** and the **Oregon Health & Science University Library**, provides unique identifiers for antibodies, model organisms, cell lines, and tools including software and databases. These IDs, called Research Resource Identifiers (RRIDs), are machine-readable and can be used to search for all papers where a

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Authors are asked to use RRIDs to cite the resources used in their research where applicable in the text, similar to a regular citation or Genbank Accession number. For antibodies, authors should include in the citation the vendor, catalogue number, and RRID both in the text upon first mention in the Methods section. For software tools and databases, please provide the name of the resource followed by the resource website, if available, and the RRID. For model organisms, the RRID alone is sufficient.

Additionally, authors must include the RRIDs in the list of keywords associated with the manuscript.

To Obtain Research Resource Identifiers (RRIDs)

1. Use the Resource Identification Portal, created by the Resource Identification Initiative Working Group.
2. Search for the research resource (please see the section titled “Search Features and Tips” for more information).
3. Click on the “Cite This” button to obtain the citation and insert the citation into the manuscript text.

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If any difficulties in obtaining identifiers arise, please contact **rii-help@scicrunch.org** for assistance.

Example Citations

Antibodies: "Wnt3 was localized using a rabbit polyclonal antibody C64F2 against Wnt3 (Cell Signaling Technology, Cat# 2721S, RRID: AB_2215411)"

Model Organisms: "Experiments were conducted in c. elegans strain SP304 (RRID:CGC_SP304)"

Cell lines: "Experiments were conducted in PC12 CLS cells (CLS Cat# 500311/p701_PC-12, RRID:CVCL_0481)"

Tools, Software, and Databases: "Image analysis was conducted with CellProfiler Image Analysis Software, V2.0 (http://www.cellprofiler.org, RRID:nif-0000-00280)"

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If you would like to send suggestions for artwork related to your manuscript to be considered to appear on the cover of the journal, **please follow these guidelines**.

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The acceptance criteria for all papers are the quality and originality of the research and its significance to journal readership. Manuscripts are single-blind peer reviewed. Papers will only be sent to review if the Editor-in-Chief determines that the paper meets the appropriate quality and relevance requirements.

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Patient anonymity should be preserved. When detailed descriptions, photographs, or videos of faces or identifiable body parts are used that may allow identification, authors should obtain the individual's free prior informed consent. Authors do not need to provide a copy of the consent form to the publisher; however, in signing the author license to publish, authors are required to confirm that consent has been obtained. Wiley has a **standard patient consent form** available for use. Where photographs are used they need to be cropped sufficiently to prevent human subjects being recognized; black eye bars should not be used as they do not sufficiently protect an individual’s identity).

Animal Studies

A statement indicating that the protocol and procedures employed were ethically reviewed and approved, as well as the name of the body giving approval, must be included in the Methods section of the manuscript. Authors are encouraged to adhere to animal research reporting standards, for example the **ARRIVE guidelines** for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

- US authors should cite compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.
- UK authors should conform to UK legislation under the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039).
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Clinical Trial Registration

The Journal will only consider for publication clinical trials that have been registered *prospectively* in a publicly accessible database. (Please note that the International Committee of Medical Journal Editors require registration of clinical trials prior to enrollment of the first participant. Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801) states that Clinical Trials need to be registered within 21 days of enrollment of the first participant).

Studies that do not meet the above requirements for potentially valid reasons (e.g., manuscripts reporting on long-term outcomes of trials initiated in the distant past) may be considered after special deliberation among the Associate Editors and the Editor in Chief.

The registration database, the registration number, and the registration date must be noted at the end of the abstract as well as in the text of the Materials and Methods.

In addition, the cover letter should include:

1. A link to the study registration in the registration database
2. An explicit statement on when the study was registered and when enrollment commenced.

Research Reporting Guidelines

Accurate and complete reporting enables readers to fully appraise research, replicate it, and use it. Authors are required to adhere to recognised research reporting standards. The EQUATOR Network collects more than 370 reporting guidelines for many study types, including for:

- Randomised trials : CONSORT
  Clinical trials should be reported using the CONSORT guidelines. A CONSORT checklist should also be included in the submission material. If your study is a randomized clinical trial, you will need to fill in all sections of the CONSORT Checklist. If your study is not a randomized trial, not all sections of the checklist might apply to your manuscript, in which case you simply fill in N/A.

- Observational studies : STROBE
- Systematic reviews : PRISMA
- Case reports : CARE
- Qualitative research : SRQR
- Diagnostic / prognostic studies : STARD
- Quality improvement studies : SQUIRE
- Economic evaluations : CHEERS
- Animal pre-clinical studies : ARRIVE
- Study protocols : SPIRIT
- Clinical practice guidelines : AGREE

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- Future of Research Communications and e-Scholarship (FORCE11)
- National Research Council's Institute for Laboratory Animal Research guidelines
- The Gold Standard Publication Checklist from Hooijmans and colleagues
- Minimum Information Guidelines from Diverse Bioscience Communities (MIBBI) website
- FAIRsharing website

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Upon its first use in the title, abstract, and text, the common name of a species should be followed by the scientific name (genus, species, and authority) in parentheses. For well-known species, however, scientific names may be omitted from article titles. If no common name exists in English, only the scientific name should be used.

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- EMBL Nucleotide Archive: ebi.ac.uk/ena
- GenBank: www.ncbi.nlm.nih.gov/genbank

Proteins sequence data should be submitted to either of the following repositories:

- Protein Information Resource (PIR): pir.georgetown.edu
- SWISS-PROT: expasy.ch/sprot/sprot-top

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- Inorganic compounds: *Fachinformationszentrum Karlsruhe* (FIZ; fiz-karlsruhe.de).
- Proteins and nucleic acids: *Protein Data Bank* (rcsb.org/pdb).
- NMR spectroscopy data: *BioMagResBank* (bmrb.wisc.edu).

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The journal requires that all 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 directly 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. The existence of a conflict of interest does not preclude publication. If the authors have no conflict of interest to declare, they must also state this at submission. It is the responsibility of the corresponding author to review this policy with all authors and collectively to disclose with the submission ALL pertinent commercial and other relationships. Please provide adequate detail that allows reproduction of the analyses after access to the original data. Be explicit about the basis of computation of the sample size and the power calculations. For clinical trials, Intent-to-treat analyses are expected, and may be supplemented by per-protocol or other types of analyses, if applicable.

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3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Access and sharing

When the article is published online:

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