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Dissertação de Mestrado

Parâmetros salivares em pacientes com Diabetes Mellitus e sua relação com atividade de cárie.

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Brasília, 29 de julho de 2021

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Dissertação apresentada ao Programa de Pós Graduação em Odontologia da Faculdade de Ciências da Saúde da Universidade de Brasília, como requisito parcial à obtenção do título de Mestre em Odontologia.

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“Consagre ao Senhor tudo o que você faz, e os seus planos serão bem sucedidos”.

Provérbios 16:3

RESUMO

Introdução: Sugere-se que pacientes com Diabetes Mellitus (DM) apresentam risco aumentado de doença cárie e a plausibilidade biológica foi creditada a parâmetros salivares alterados, como pH salivar, fluxo salivar e capacidade tampão. No entanto, a ocorrência de cárie no diabetes tipo 2 (DM2) não é totalmente compreendida até o momento, principalmente na população idosa. **Objetivo:** Avaliar a associação entre parâmetros salivares e cárie, em indivíduos portadores de Diabetes Mellitus. **Métodos:** Dois estudos foram realizados: uma revisão sistemática e um estudo transversal. A revisão sistemática foi realizada de acordo com o PRISMA e a busca foi realizada em oito bases de dados, bem como na literatura cinzenta. A qualidade metodológica foi avaliada usando as ferramentas do Joanna Briggs Institute para estudos observacionais. Sete metanálises foram realizadas. O estudo transversal incluiu 54 indivíduos, dos quais 35 pertenciam ao grupo controle sistemicamente saudável e 19 ao grupo DM2. Os indivíduos foram classificados como controlados ou não controlados (HbA1c≥6,5 e / ou FBG> 100). Os parâmetros clínicos examinados foram: cárie coronária ativa e cárie radicular, pH salivar, fluxo salivar, capacidade tampão e amilase salivar. **Resultados:** 22 estudos observacionais foram incluídos, representando um total de 1.202 e 946 pacientes com DM e controles saudáveis. Indivíduos com DM apresentaram menores taxas de fluxo salivar não estimulado e estimulado quando comparados aos controles. A ureia salivar foi significativamente maior no DM em comparação aos controles. No estudo transversal, o grupo DM2 apresentou maior número de lesões de cárie radicular do que indivíduos sem DM2. O fluxo salivar não estimulado foi menor nos indivíduos com DM2 não controlado, assim como o pH salivar. Nos modelos de regressão, diabéticos têm 2,25 vezes mais chance de apresentar aumento do número de superfícies com cárie radicular (p= 0,04). **Conclusão:** Parâmetros salivares como a redução do fluxo salivar não estimulado não explicam por si só o maior número de lesões de cárie radicular em pacientes com DM2. Mais fatores, como composição salivar, hábitos de higiene, dieta e microbiota estão envolvidos para explicar o risco aumentado de cárie radicular nesses indivíduos.

PALAVRAS-CHAVE: Diabetes mellitus, saliva, fluxo salivar, tampão, cálcio, fósforo, ureia, cárie dentária.

ABSTRACT

Introduction: It has been suggested that diabetic patients are at increased risk of dental caries and it was credited to altered salivary parameters, such as salivary pH, salivary flow and buffering capacity. However, the occurrence of dental caries in type 2 diabetes (T2D) is not fully understood so far, particularly in the elderly population. **Objective:** To evaluate the association between salivary parameters and caries in individuals with Diabetes Mellitus. **Methods:** Two studies were developed: a systematic review and a cross-sectional study. The systematic review was carried out according to PRISMA and the search was carried out in eight databases, as well as in the gray literature. The methodological quality assessment was assessed using the Joanna Briggs Institute critical assessment tools for observational studies. Seven meta-analyses were performed. The cross-sectional study included 54 individuals, from which 35 were systemically healthy and 19 had T2D. Subjects were then classified as controlled or uncontrolled (HbA1c ≥ 6.5 and/or FBG>100). The clinical parameters examined were: active coronal caries and root caries, salivary pH, salivary flow, buffering capacity, and salivary amylase. **Results:** 22 observational studies were included, representing a total of 1,202 and 946 T2D patients and healthy controls. Individuals with DM had lower rates of unstimulated and stimulated salivary flow compared to controls. Salivary urea was significantly higher in T2D compared to controls. In the cross-sectional study, the T2D group had a greater number of root caries lesions than individuals without T2D. Unstimulated salivary flow were lower in individuals with uncontrolled DM, as well as the salivary pH. In the regression models, diabetics are 2.25 times more likely to have an increased number of surfaces with root caries. **Conclusion:** Studies suggest that individuals with T2D have altered salivary parameters when compared to individuals without DM. Salivary parameters such as the reduced unstimulated salivary flow do not explain by themselves the greater number of root caries lesions in patients with T2D. More factors, such as salivary composition, hygiene habits, diet and microbiota are involved to explain the increased risk of root caries in these individuals that need to be explored.

KEYWORDS: Diabetes mellitus, saliva, salivary flow, buffer, calcium, phosphorus, urea, Dental caries.

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CAPÍTULO 3

LISTA DE ABREVIATURAS E SIGLAS

BP	Bleeding on Probing
C	Grupo Controle
Ca	Cálcio
DM	Diabetes Mellitus
DM1	Diabetes Mellitus tipo 1
DM2	Diabetes Mellitus tipo 2 (em português)
DMP	Grupo diabetes e doença periodontal
FBG	Glicose capilar
FCWBG	Glicose capilar (em inglês)
FSEG	Glicose Sérica
FSLG	Glicose Salivar
GR	Recessão gengival (em inglês)
IL	Nível de inserção (em inglês)
IgA	Imunoglobulina A
HbA1c	Hemoglobina glicada
NA	Não aplicável
P	Fósforo
pH	Potencial de hidrogênio
S-IgA	Imunoglogulina salivar
US	Saliva em repouso
U	Saliva estimulada
T1D	Diabetes Mellitus tipo 1 (em inglês)
T2D	Diabetes Mellitus tipo 2 (em inglês)
PC	Controle metabólico inadequado
PD	Profundidade de sondagem (em inglês)
PI	Índice de placa (em inglês)
WC	Bom controle metabólico

SUMÁRIO

1.CAPÍTULO 1 - INTRODUÇÃO, REVISÃO DE LITERATURA E OBJETIVOS

1.1 INTRODUÇÃO

A medida que a expectativa de vida da população aumenta, observa-se incidência de doenças crônicas, como a Diabetes mellitus (DM)(1). A DM é uma doença metabólica de várias causas, caracterizado por hiperglicemia crônica, que, ao longo do tempo, pode causar danos aos vasos sanguíneos. Devido a importância do sistema circulatório na nutrição sanguínea de todo o corpo, esses danos aos vasos sanguíneos podem trazer consequências ao coração (infarto do miocárdio), cérebro (acidente vascular), olhos (retinopatia diabética), rins (nefropatia diabética) e nervos (neuropatia diabética). Além disso o excesso de glicose pode causar danos ao sistema imunológico, devido a diminuição da eficácia dos glóbulos brancos pela hiperglicemia (2). Nas últimas décadas, a prevalência da doença vem aumentando e já atinge cerca de 422 milhões de pessoas no mundo, principalmente em países de baixa e média renda. Por configurar um problema de saúde pública, existe uma meta globalmente acordada de interromper o aumento do diabetes e da obesidade até 2025 (1).

Manifestações bucais são comuns na DM. O quadro de hiperglicemia induz respostas inflamatórias, o que contribui para a degradação sistêmica dos tecidos conjuntivos, incluindo os periodontais, e possível diminuição do pH bucal. Outras manifestações podem ocorrer relacionadas à composição e funções salivares, como por exemplo a diminuição do fluxo salivar (3). Quando o fluxo salivar diminui, diversas alterações bucais podem ocorrer, tais como, o aumento da concentração de mucina e glicose, que resultam na mudança do ambiente bucal, levando à estimulação de uma atividade cariogênica (4). Além disso a hiperglicemia sanguínea se traduz em maiores concentrações de glicose nos fluidos bucais, podendo favorecer a proliferação de microrganismos fermentadores, o que pode levar pacientes diabéticos a ter maior risco de desenvolver cárie (4).

Segundo uma revisão sistemática de Wang *et al*, a prevalência e gravidade da doença cárie entre crianças e adolescentes com diabetes é maior do que na população em geral (5). Porém, a relação entre diabetes e doença cárie entre adultos recebeu bem menos atenção até agora, apesar do fato de ambas as doenças estarem associadas ao mesmo fator causal: a ingestão de carboidratos.

Sendo a saliva um fluido biológico complexo que contém vários compostos que colaboram para prevenir a cárie dentária por lavagem mecânica, função antimicrobiana, remineralização e regulação do pH bucal por sua capacidade de tamponamento (6), a alteração do fluxo e composição salivar observada em pacientes diabéticos descompensados, teoricamente, podem comprometer essa função protetiva e predispor os mesmos à doença cárie. Ao contrário, estudos mostram que pacientes diabéticos com bom controle glicêmico, e com baixa ingestão de açúcares, consequentemente, têm menor chance de desenvolver cárie (5). Apesar de várias teorias, faltam evidências e estudos mais consistentes que confirmem que as alterações no fluxo e composição salivar em pacientes diabéticos estejam diretamente relacionadas a maior ou menor desenvolvimento de lesões de cárie. O objetivo deste estudo é, portanto, dissertar sobre o grau de evidências científicas que se tem sobre os parâmetros salivares em indivíduos com Diabetes Mellitus tipo 2 (DM2) e sua possível relação com cárie dentária, por meio de um estudo transversal e de uma revisão sistemática da literatura.

1.2 REVISÃO DE LITERATURA

1.2.1 Saliva

A saliva é um fluido diluído, sendo a água responsável por 99% de sua composição, e o 1% restante é formado por componentes inorgânicos: minerais como cálcio e fosfato, e orgânicos: proteínas e enzimas (7). Na composição da saliva coletada na cavidade bucal, podemos também encontrar secreções de todas as glândulas, células epiteliais bucais descamadas, microrganismos e

seus produtos, leucócitos, constituintes séricos, fluido crevicular gengival e componentes da dieta. A saliva é secretada pelas glândulas salivares na cavidade bucal, de pH neutro que varia entre 6,2 a 7,6, com 6,7 sendo o pH médio. Em repouso, o pH da boca fica normalmente acima de 6,3. Na cavidade bucal, o pH é mantido próximo à neutralidade (6,7-7,3) pela saliva (7). Nas últimas três décadas, estudos foram desenvolvidos com o objetivo de utilizar a saliva em abordagens diagnósticas para monitorar as doenças bucais (8). Como a maioria dos componentes do sangue podem ser encontrados também na saliva, o avanço da biotecnologia associado a diagnósticos salivares tornou possível obter vários diagnósticos relacionados ao sistema fisiológico, utilizando a saliva da cavidade bucal. Por conseguinte, a saliva pode conter biomarcadores fisiológicos do corpo, e servir para o monitoramento da saúde bucal e também sistêmica e refletir variações nutricionais, metabólicas, endócrinas e emocional (8).

1.2.2 Hipossalivação e Diabetes Melitus (DM)

Enquanto a xerostomia é uma queixa subjetiva de boca seca, a hipossalivação é uma redução objetiva do fluxo salivar. Quando as taxas de fluxo salivar estão abaixo de 0,1 ml/min em repouso, ou 0,7 ml/min quando estimulada, considera-se um quadro de hipossalivação (9).

Inúmeros fatores podem induzir distúrbios salivares em pacientes com DM, como desordens sistêmicas, o envelhecimento, radioterapia de cabeça e pescoço, bem como várias drogas. Tanto a diabetes tipo 1 (DM1) como diabetes tipo 2 (DM2), foram previamente associadas à xerostomia (10). Também existem estudos que relatam diminuição do fluxo salivar em pacientes com DM em relação a pacientes sem DM (3,10,11). Danos no parênquima glandular, alterações na microcirculação das glândulas salivares, desidratação e distúrbios no controle glicêmico são hipóteses que podem explicar esses problemas (10).

Corroborando com estudos prévios, uma revisão sistemática realizada por López-Pinto et al, demonstrou uma variação considerável na prevalência de xerostomia e taxas de fluxo salivar entre a população com DM e pacientes do grupo controle sem DM. A maioria dos estudos encontrou maior prevalência de

xerostomia e menores taxas de fluxo salivar no DM em relação aos grupos controles saudáveis. Porém, devido ao alto grau de heterogeneidade em relação aos tipos de DM, diagnóstico de DM, idade dos pacientes e tipos e técnicas de coleta de fluxo salivar, foi difícil comparar os estudos. Além disso, a avaliação da qualidade mostrou a baixa qualidade dos estudos existentes, tornando os resultados da revisão sistemática inconsistentes, sendo necessário novos estudos que usem definições mais precisas e atuais sobre a determinação e o diagnóstico de pacientes com DM e a coleta da taxa de fluxo salivar. Esses novos estudos devem compreender pacientes com DM e sem DM que não possuam fatores confundidores, como o uso de medicamentos e doenças associados à xerostomia e hipossalivação (que não sejam DM) e idade avançada, pois a redução do fluxo salivar nem sempre é patológica (3).

1.2.3 Parâmetros salivares qualitativos em DM

1.2.3.1 Conteúdo orgânico

Além de alteração no fluxo salivar, a DM pode estar associada a alterações nos componentes salivares que podem afetar a incidência, sinais e gravidade das alterações bucais em pacientes(12,13).

1.2.3.1.1 Glicose salivar

Uma revisão sistemática com metanálise realizada por Naseri *et al*, comparou o nível de glicose salivar de pacientes com DM e controles saudáveis, sem discrepância de idade entre os grupos. Foram analisados 20 estudos que, na estimativa combinada, mostraram que o nível de glicose salivar foi significativamente maior no grupo DM do que nos controles saudáveis (MD = 6,77 mg/dL). Quando subdivididos em grupos com base na condição de amostragem da saliva, os estudos mostraram que o nível de glicose salivar, tanto nas condições de jejum quanto não-jejum, era significativamente maior em pacientes com DM do que nos controles saudáveis ([MD = 6,23 mg / dL] e [MD = 6,70 mg/dL], respectivamente), indicando alta heterogeneidade em dois subgrupos (14). Essa grande diferença na média de glicose salivar entre os

grupos, reflete o fato de que, a maioria dos estudos, trabalharam com grupos DM com glicemia elevada (não controlados). Corroborando com o dito anteriormente, dez destes estudos mostraram uma correlação significante entre os níveis de glicose salivar e glicose sérica nos pacientes com DM (P < 0,001). Outros 8 estudos encontraram uma correlação significativa entre os níveis de glicose salivar e sérica não só em pacientes com diabetes, como também em controles saudáveis (15–22). Essa meta-análise também mostrou uma correlação significativa entre os níveis salivar e sérico de hemoglobina glicada (HbA1c) em pacientes diabéticos, pois quatro estudos confirmaram e apenas um estudo não (14).

Esses resultados são animadores, pois demonstram que a glicose salivar pode ser usada para fins de diagnóstico e monitoramento da DM. Nessas condições, o conteúdo de glicose na saliva atraiu a atenção de pesquisas para elaboração de métodos de monitoramento de glicose, devido as vantagens inerentes a amostras de saliva, como não invasividade e facilidade de coleta. No estudo de Dominguez *et al*, por exemplo, foi avaliado o desempenho de um colorímetro de baixo custo para quantificar a concentração de glicose nas amostras salivares em jejum de 41 voluntários. Os resultados da concentração de glicose salivar obtidos para o grupo diabético foram significativamente maiores do que para o grupo controle (23). Outro estudo recente de Ephraim *et al* compararam o desempenho diagnóstico e a correlação entre glicose salivar, sérica e capilar no sangue de pacientes com DM (recém diagnosticados) e um grupo controle. Os níveis de glicose salivar em jejum, glicemia sérica em jejum e glicemia capilar em jejum foram analisados para cada participante. Neste estudo, também foi possível observar que os níveis médios de glicose nos 3 tipos de testes foram significativamente maiores entre os casos em comparação aos controles. Houve uma diferença média significativa entre os níveis de glicose salivar vs. glicose sanguínea e glicose salivar em jejum vs. glicemia capilar total em jejum, mas não os níveis de glicose sérica em jejum vs. glicemia capilar total em jejum nos dois casos e controles. Foi observada correlação positiva entre glicose salivar em jejum e glicose sérica em jejum (r = 0,89) e glicemia capilar total em jejum (r = 0,87). No valor de corte> 6,8 mmol / l para glicose sérica em jejum, observou-se sensibilidade de 99%, especificidade de 100,0% e área sob

a curva (AUC) de 98,8% para predizer DM, enquanto sensibilidade de 80%, especificidade de 95% e AUC de 91,0% foram observadas para glicose salivar em jejum com um valor de corte> 0,5 mmol / l. No valor de corte> 6,9 mmol / l para glicemia capilar total em jejum, foram detectadas sensibilidade de 100,0%, especificidade de 100,0% e AUC de 100. No entanto, a glicose salivar não gera precisão diagnóstica e preditiva suficiente em comparação com a glicemia capilar total, que é menos invasiva (22).

1.2.3.1.2 Glicose salivar x Cárie

Sabendo que bactérias tem como fonte de energia a glicose, e considerando que boa parte das bactérias salivares são acidogênicas, uma saliva com níveis de glicose alterados pela hiperglicemia sanguínea pode favorecer o desenvolvimento dessas bactérias, provocando um nível aumentado de síntese bacteriana de metabólitos ácidos, e consequentemente diminuindo o pH.

De fato, a presença de carboidratos na dieta é bem reconhecida como um estímulo para o pH salivar mais baixo devido ao metabolismo incompleto dos carboidratos pelas bactérias acidogênicas bucais (24). A acidificação é proposta para interferir na reprodução bacteriana, alterando a frequência relativa das espécies bacterianas e a contagem do microbioma bucal. Esta proposta é consistente com a redução do crescimento bacteriano bucal em condições de pH mais baixo (25,26).

Sabe-se que a acidificação salivar é um fator importante no desenvolvimento de cárie dentária e gengivite porque altera o microbioma bucal para favorecer espécies bacterianas associadas à cárie (25,27). Uma alta concentração de glicose salivar também parece aumentar o risco de erosão dentária, cárie dentária e gengivite.

Almusawi *et al* propõem em seu estudo que pacientes com DM2 apresentam alto risco de doença cárie, que está diretamente associada a FBG (Glicemia de jejum), HbA1c (hemoglobina glicada) e glicose salivar (28). Um estudo de Godson *et al*, onde foram comparadas amostras de saliva e dados

clínicos de pacientes kuwaitianos, com objetivo de comparar aos níveis séricos de glicose desses adolescentes, observou que o grupo de adolescentes com maior percentual de lesões de cárie também possuíam taxa de glicose salivar alta quando comparados com grupo de glicose salivar normal. Nesse mesmo estudo, houve uma diminuição da carga bacteriana total de quase todas as espécies avaliadas por hibridização DNA-DNA *checkerboard* em amostras com glicose salivar aumentada, o que sugere que altos níveis de glicose salivar sejam capazes de afetar a microbiota oral, sendo necessários estudos mais consistentes (4). Porém, o estudo não registrou dados sobre a dieta e frequência alimentar desses adolescentes. Sabendo que DM2 e doença cárie, como dito anteriormente, dividem o mesmo fator causal (o alto consumo de carboidratos), não é possível relacionar as lesões de cárie apenas ao elevado índice de glicose salivar.

1.2.3.1.3 Alterações e controle do pH da saliva em diabéticos

A saliva mantém o pH próximo a neutralidade por dois mecanismos. Primeiro, o fluxo salivar remove os carboidratos que poderiam ser metabolizados pelas bactérias, e consequentemente, o ácido produzido pelas bactérias é eliminado. Segundo, a saliva neutraliza a acidez formada a partir de alimentos, bebidas e da atividade microbiana, devido a sua capacidade de tamponamento (7). A capacidade tampão da saliva é a propriedade de a saliva manter o seu pH constante a 6,9-7,0, através de seus tampões, mucinato/mucina, bicarbonato/ ácido carbônico e hidrogenofosfato /ácido fosfórico, que bloqueiam o excesso de ácidos e de bases. A anidrase carbônica faz tamponamento da saliva, catalisando a reação entre CO2 e H2O que se dissociam em HCO3, neutralizando os ácidos produzidos pelas bactérias durante o processo de fermentação (29). A capacidade tamponante da saliva é um importante fator de resistência à doença cárie, pois age neutralizando os ácidos bucais (30). O reduzido fluxo salivar, que geralmente está associado a uma baixa capacidade tamponante, pode contribuir para infecções da mucosa bucal (31). Outra avaliação interessante da capacidade tampão da saliva foi feita no trabalho de Alves *et al* (22), através da dissolução de glóbulos de sacarose sobre a língua. O pH salivar tende-se a reduzir com a produção de ácidos a partir da

fermentação de açúcares da sacarose. No entanto o sistema tampão regulariza o pH em poucos minutos, provocando o aumento do pH salivar em cerca de 20 minutos após a dissolução dos glóbulos. Muitas bactérias necessitam de um pH específico para seu crescimento máximo, a capacidade tampão da saliva evita a colonização da boca por microrganismos potencialmente patogênicas, por negar-lhes a otimização das condições ambientais. Os microrganismos da placa podem produzir ácido a partir de açúcares, os quais, não sendo rapidamente tamponados e limpos pela saliva, podem desmineralizar o esmalte (32). Resíduos carregados negativamente sobre as proteínas salivares funcionam também como tampões; um peptídeo salivar, conhecido como sialina, tem um importante papel no aumento do pH da placa dental após exposição a carboidratos fermentáveis. Sendo assim, a capacidade da saliva como um tampão ácido é muito importante, porque a saliva e o pH da placa são geralmente baixos nas lesões de cárie ativas. A manutenção de pH é um aspecto essencial para a prevenção de lesões relacionadas à cárie. No estudo feito por Kaur *et al* (33) observou-se que pacientes com baixa ou nenhuma atividade de cárie possuíam um pH neutro de 7, e indivíduos com lesões cariosas apresentavam um pH abaixo do pH crítico, 5,5.

Alterações no pH salivar em indivíduos com DM também são comumente relatadas e podem representar um risco maior para o desenvolvimento de lesões cariosas (34,35). Na hiperglicemia, uma deficiência de insulina, que é um hormônio fundamental para a regulação dos níveis de açúcar no sangue, resulta em um aumento de outros hormônios, como glucagon e cortisol, que induzem áreas do corpo que geralmente degradam os açúcares por meio da ação da insulina, começam a degradar as gorduras. É essa “queima de gordura” para produzir energia, devido à indisponibilidade de açúcares, que acaba produzindo corpos cetônicos que se acumulam no sangue, e tornam o sangue mais ácido, com queda do pH (36).

Um ambiente acidogênico com baixo pH salivar favorece o crescimento de patógenos periodontais e de bactérias acidúricas, criando um ambiente inóspito para as bactérias bucais protetoras, que pode levar ao agravo de

doenças periodontais e desenvolvimento de lesões cariosas, que reduz ainda mais o pH salivar, levando a um ciclo repetitivo (33).

É o que Seethalakshmi *et al* demonstraram em um estudo transversal, onde comparou o pH salivar médio entre pacientes com e sem DM. Os indivíduos com DM apresentaram menor pH salivar e maior prevalência de cárie e periodontite quando comparado ao grupo de pacientes saudáveis. Isso pode ser atribuído às alterações metabólicas em pacientes com DM, resultando em pH ácido, onde há redução no nível de bicarbonatos em todos os fluidos corporais, o que leva à acidose metabólica de todos os fluidos corporais. Neste mesmo estudo pacientes com DM apresentaram aumento do escore de CPOD quando comparado ao grupo controle, tendo como hipótese explicativa a perda do mecanismo de proteção da saliva em diabéticos decorrente da redução do pH(34). Entretanto, não se pode pressupor que as perdas dentárias foram decorrentes à cárie, e não à doença periodontal preexistente. Além disso o índice CPOD não reflete atividade de cárie (apenas a história da doença), uma vez que podemos ter lesões cavitadas, porém inativas, e lesões não cavitadas, mas ativas. Também é importante salientar, que indivíduos com DM e bom controle glicêmico, quando acompanhados por nutricionistas, devem ingerir menor quantidade de açúcar e, com isso, apresentam menor chance de desenvolver a doença cárie. Como os indivíduos deste estudo não tiveram dieta analisada (com registro da frequência de consumo de carboidratos) não se pode afirmar que este aumento do escore de CPOD não esteja relacionado ao consumo excessivo de açúcar, que tenha causado tanto a DM como a cárie. Também para ignorar o efeito do ritmo circadiano na quantidade e composição da saliva, as amostras deveriam ser coletadas em um determinado horário do dia. E por fim, não discriminaram o nível de controle metabólico dos pacientes, o que impediu diferenciar as manifestações bucais entre diabéticos compensados e descompensados, sendo necessários estudos mais consistentes sobre o assunto.

1.2.3.1.4 Ureia

Segundo Chen *et al*, a ureia é o componente mais abundante (não proteico) na saliva usado por bactérias como *Streptococcus salivarius,*

Actinomyces naeslundii aparentemente por meio de sua expressão de urease (37), uma enzima que converte ureia em amônia e dióxido de carbono.

Embora a produção de amônia na placa ajude a neutralizar o ácido láctico nas lesões de cárie (38), uma revisão recente concluiu que não houve efeito benéfico na cárie (39). Para compreender melhor o metabolismo da ureia pelas bactérias bucais, amostras com ureia marcada foram analisadas por ressonância magnética nuclear que permite o rastreamento da ureia adicionada. Surpreendentemente, a ureia foi inicialmente convertida em carbamato de amônio e depois em formato e propionato, e não em amônia. Embora a conversão de ureia em carbamato de amônio tenha sido descrita antes, mesmo pela urease (40), presume-se que ela se degrade em amônia e dióxido de carbono.

Se a atividade da urease estivesse presente na boca, quantidades de ureia estariam ausentes ou em concentrações reduzidas. Isso é interessante porque pode explicar as grandes quantidades de formato na saliva e a falta de eficácia da ureia na prevenção da cárie. Porém, os presentes resultados não excluem a possibilidade de ação da urease e que a produção de amônia possa depender da quantidade de ureia adicionada. Claramente, mais trabalhos são necessários para substanciar essa nova ideia e delinear quais bactérias convertem ureia em formato e / ou quais convertem em amônia.

Outra implicação que os elevados níveis de ureia podem ter na saúde bucal está relacionado a halitose. Um estudo de Khozeimeh *et al* mostraram que a concentração de ureia salivar e ácido úrico no grupo com pessoas com halitose foi significativamente maior do que no grupo controle (41). As taxas de ureia salivar e concentração de ácido úrico para creatinina foram maiores no grupo halitose. Os resultados da avaliação do perfil salivar em pacientes com DM2 em comparação com controles saudáveis mostraram que os níveis de ureia foram significativamente mais elevados em DM do que os controles.

Vários estudos têm correlacionado o aumento de ureia salivar a DM (42–44). A comparação dos valores de ureia sérica com valores de ureia salivar mostraram que em 30 pacientes diabéticos comparados aos 30 controles adultos

saudáveis (idade e pareados por gênero), os valores de ureia sérica foram significativamente mais altos do que a ureia salivar e os níveis de ureia salivar foram maiores em pacientes diabéticos do que em controles saudáveis (45). Um estudo incluiu 60 participantes de ambos os sexos com faixa etária de 30-70 anos (30 pacientes com diabetes insulino-dependentes e 30 controles), nos quais os níveis de ureia salivar e glicose salivar foram significativamente maior em pacientes diabéticos do que no controle grupo(46).

A nefropatia diabética é uma complicação causada pelo diabetes na microvasculatura renal. Indivíduos com diabetes apresentam maior taxa de filtração glomerular ou hiperfiltração, mediada por maior relaxamento das arteríolas aferentes em comparação com as arteríolas eferentes. Isso, por sua vez, leva a um aumento do fluxo sanguíneo através do capilar glomerular, aumentando a pressão. Quando essas condições são mantidas ao longo do tempo, elas produzem hipertrofia glomerular e um aumento na superfície do capilar glomerular. Isso acarreta alterações hemodinâmicas que contribuem para o desenvolvimento e / ou progressão da doença (47,48). O aumento da ureia plasmática é um sinal básico de problemas renais. Consistente com a literatura científica, a uréia salivar é significativamente maior em pacientes diabéticos (42,49). Sabendo que a nefropatia não é incomum em pacientes com DM, a ureia salivar parece ser um marcador futuro promissor no monitoramento de pacientes com nefropatia. Além disso, como dito anteriormente, a ureia aumentada nos diabéticos poderia ser um fator protetor para doença cárie e essa variável precisa ser mais bem explorada.

1.2.3.1.5 Proteínas Salivares

Recentemente, houve um rápido aumento no número de estudos dedicados à identificação dos componentes proteicos da saliva para melhorar o diagnóstico e o monitoramento de diversas doenças. Consequentemente, a busca por biomarcadores salivares iniciou a catalogação do proteoma salivar humano tanto de indivíduos saudáveis quanto de indivíduos com doenças bucais e sistêmicas. A IgA secretora (s-IgA) é um agente de defesa antimicrobiana contra patógenos de primeira linha do sistema imunológico e desempenha um

papel importante na saúde bucal. A amilase é uma enzima dependente de cálcio encontrada na saliva que quebra o amido em maltose e dextrina (50).

Tem se observado em pacientes diabéticos uma maior penetração de proteínas na saliva, como a amilase, devido a alterações na permeabilidade da membrana base das glândulas salivares e alguns estudos têm mostrado uma maior expressão de receptores de amilase no diabetes (51). Kim *et a.l* (52) e de Prathibha *et a.l* (53) mostraram níveis mais baixos de amilase em indivíduos com DM, enquanto os achados anteriores (54–56) relataram maiores quantidades de amilase salivar nos participantes. Alguns outros estudos não declararam alterações nas quantidades de amilase salivar em pacientes com diabetes (57,58). Uma revisão sistemática demostrou em sua metanálise não haver diferenças nas taxas de amilase salivar entre o grupo DM e controle em jejum, mas em situação de não jejum, foi encontrado diferença, o que pode explicar as diferenças encontradas entre os estudos (12). Porém, esta revisão incluia em sua amostra estudos com pacientes jóvens e idosos, tornando a idade um fator confundidor.

Kheirmand *et al* (59) demonstraram que os níveis salivares de IgA e amilase estão associados ao DM. Além disso, os níveis de s-IgA estão associados ao DM2 em pacientes com candidíase bucal, placa branca, abscessos ou manifestações de xerostomia, enquanto os níveis de amilase salivar estão associados ao DM2 em pacientes com candidíase bucal ou manifestações de candidíase eritematosa. Esses achados estão de acordo com os de estudos anteriores (56,60,61). Por outro lado, Salles *et al*. (62) relataram uma concentração menor de s-IgA em pacientes diabéticos em comparação com não diabéticos, o que provavelmente está relacionado à distribuição não homogênea por gênero e ao uso de pacientes com DM1 e DM2 em suas pesquisas. Mas ainda existem poucos estudos correlacionando proteínas salivares a cárie na população adulta e idosa. Torna-se importante incluir essas variáveis em estudos futuros, para melhor entender o papel desses componentes na saúde oral.

1.2.3.2 Conteúdo inorgânico

Na cavidade bucal, os eletrólitos salivares desempenham papel relevante na proteção individual contra a doença cárie. Esses eletrólitos mantêm a saliva supersaturada em relação à hidroxiapatita e exercem uma influência positiva na reparação do esmalte dentário. Do ponto de vista bioquímico, existem fortes evidências sobre as propriedades físico-químicas da saliva e seu efeito anticárie (31). No entanto, evidências limitadas de estudos clínicos apoiam o efeito protetor dos eletrólitos salivares de ocorrência natural (63).

À medida que os ácidos se acumulam na fase fluida do biofilme a partir do metabolismo de carboidratos pela microbiota, o pH cai até o ponto em que as condições na interface biofilme-tecidos dentários tornam-se subsaturados, e o ácido desmineraliza parcialmente a camada superficial do dente (64). A perda de mineral leva ao aumento da porosidade, alargamento dos espaços entre os cristais de esmalte e amolecimento da superfície, o que permite que os ácidos se difundam mais profundamente no dente, resultando em desmineralização do mineral abaixo da superfície (subsuperfície desmineralização). O acúmulo de produtos da reação, principalmente cálcio e fosfato, da dissolução da superfície aumentam o grau de saturação e pode proteger parcialmente a camada superficial de desmineralização (65).

1.2.3.2.1 Cálcio e Fósforo salivar X DM

O equilíbrio entre desmineralização e remineralização depende da concentração de cálcio e fósforo salivares. Esses íons devem ser saturados em saliva para causar um efeito na desmineralização e remineralização (66). Uma vez que os açúcares são eliminados da boca ao engolir e diluídos na saliva, os ácidos do biofilme podem ser neutralizados pela ação tamponante da saliva. O pH do biofilme e fluido retornam à neutralidade e se torna suficientemente saturado com íons de cálcio, fosfato e flúor. O principal objetivo é manter a homeostase mineral das superfícies dentais. Como dentes são frequentemente expostos a condições ácidas do biofilme ou ácidos dietéticos, a capacidade de remineralizar é essencial para manter a integridade do dente. Saliva é essencial para a preservação da saúde dos dentes, fornecendo os minerais necessários para a remineralização.

Pacientes com DM podem ter seus níveis salivares de cálcio afetados, pois a secreção de insulina é um processo cálcio-dependente que faz com que os pacientes com DM2 tenham baixos níveis de insulina, diminuindo os níveis séricos de cálcio, e essa redução no soro reflete nas regras salivares. Além disso, outro estudo descobriu que um aumento na glicose sanguínea aumenta a excreção urinária de cálcio e fósforo, que é proporcional ao grau de glicosúria (67).Portanto, para melhor entendermos a relação da cárie com DM, a avaliação de níveis de íons salivares torna-se imprescindível. Devido as contradições encontradas na literatura sobre essas possíveis alterações em DM, há necessidades de novos estudos com amostras maiores, exames complementares e com metodologia semelhante. Porém, são poucos os estudos que avaliam esses parâmetros em diabéticos e as evidências ainda são pouco conclusivas.

1.3 JUSTIFICATIVA

Diante do exposto, em relação à DM e todas as possíveis alterações salivares, torna-se necessária a realização de um estudo para maior compreensão da influência do DM nas alterações salivares e suas respectivas correlações com as prevalência e extensão de cárie em comparação aos indivíduos saudáveis, principalmente em adultos. Este estudo inovador tem potencial de impacto no manejo, tratamento e na difusão do conhecimento sobre a saúde bucal de pacientes com DM.

1.4 OBJETIVOS

1.4.1 Objetivo Geral

Avaliar os parâmetros salivares de indivíduos com diabetes e sua possível relação com doença cárie.

1.4.2 Objetivos Específicos

1) Meta-analisar as evidências científicas dos estudos dos parâmetros salivares em DM quando comparados a pacientes sem DM (Artigo 1);
2) Comparar taxas de fluxo salivar, tampão, cálcio, fosfato, ureia, e pH salivar em pacientes adultos e idosos com e sem diabetes (Artigos 1);
3) Comparar o fluxo salivar estimulado e em repouso em pacientes com e sem atividade de cárie, comparando-as com a presença de DM (Artigo 2);
4) Comparar o pH salivar e a capacidade tampão em pacientes com e sem atividade de cárie, comparando-as com a presença de DM (Artigo 2);
5) Avaliar a presença de alterações salivares e correlacioná-la ao DM e ao nível de controle glicêmico a partir da concentração da hemoglobina glicosilada (HbA1c) e glicemia em jejum (Artigos 1 e 2).

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2. CAPÍTULO 2- SALIVARY BIOCHEMISTRY OF INDIVIDUALS WITH TYPE 1 OR 2 DIABETES MELLITUS: A SYSTEMATIC REVIEW AND METANALYSIS

2.1 ABSTRACT

Introduction: It is known that hyperglycemia causes several oral changes, including qualitative and quantitative changes in saliva. Due to the importance of saliva in maintaining oral health, the use of several physical, biochemical and microbiological salivary parameters in clinical dental diagnosis has become more common.

Objectives: This study aimed to compare whether salivary parameters, such as salivary flow, ionic composition, pH, buffer capacity, are altered in adults with Diabetes Mellitus (DM) when compared to individuals without DM.

Methodology: This systematic review was performed according to PRISMA and the search was performed in eight databases, as well as gray literature. The risk of bias was assessed using the Joanna Briggs Institute critical assessment tools for observational studies. Seven meta-analyses were performed. Results: Of the 5816 titles retrieved, 22 observational studies were included, representing a total of 1,202 and 946 patients with type 2 DM and healthy controls, respectively, aged between 30 and 79 years and most men (62%). Individuals with DM had lower unstimulated (p=0,0005) and stimulated (p=0,005) salivary flow rates when compared to controls. Salivary urea was significantly higher in DM compared to controls (p=0.05). In the other parameters, no significant differences were found

Conclusions: T2D (type 2 Diabetes) individuals presented reduced stimulated and non-stimulated salivary flow and altered biochemical parameters when compared with individuals without DM, including higher salivary urea. The salivary pH, buffering capacity, calcium and phosphorus concentrations were similar between groups. Regarding DM1, there is not enough data in adults to answer the research question.

2.2 INTRODUCTION

Chronic hyperglycemia and frequent disorders of carbohydrate metabolism in individuals with Diabetes Mellitus (DM) are associated with obesity, protein and electrolyte disorders, as well as with several complications (1), such as neuropathy and microvascular abnormalities with endothelial dysfunction and deterioration of the microcirculation. Those complications of DM has been linked to changes in the flow and composition of saliva (2,3).

Among the oral manifestations of DM, the salivary changes stand out, as it can be linked to environmental changes that prompt other oral diseases. As an example, changes in the salivary flow rate, pH, or buffering capacity may increase the risk of either dental caries or fungal infections in the oral mucosa. In addition, salivary immunoglobulins are important factor as the first line of defense against pathogens that colonize and invade mucous surfaces (4). Due to the importance of saliva in maintaining oral health, the use of several physical, chemical, and microbiological salivary parameters in clinical dental diagnosis has become increasingly widespread (5).

Nevertheless, to establish a correlation between oral diseases and salivary parameters, it is necessary to distinguish normal parameters and altered ones. Although there is evidence that individuals with DM have a lower salivary flow rate than individuals without DM (6), advances in investigations of salivary biochemical parameters are necessary to understand whether DM changes the quality of saliva, as well as consequences of these changes, can impact the oral health of these individuals. It is well established in the literature that there is a reflection of DM in some salivary components, such as the increase in salivary levels of glucose, total proteins, and amylase (7,8). However, in order to raise hypotheses about the impact that these changes may have on the oral health of this population, we need to evaluate other components that play an important role in the salivary function. In addition, only recent studies have directed attention to the elderly population and, consequently, to type 2 DM (T2D).

Based on these findings, we assessed whether the salivary, physical (salivary flow) and biochemical parameters (phosphorus and calcium content,

urea, buffering capacity, and pH), differs between individuals with and without DM. The aim of this study was to compare the salivary parameters of adults and the elders with and without DM, as well as comparing the type 1 and type 2DM.

2.3 METHODS

This study was conducted according to the declaration of preferred report items (PRISMA) for a systematic review and meta-analysis of diagnostic test accuracy studies(9) and recommendations from the Cochrane collaboration. The protocol was registered at the PROSPERO International prospective register of systematic reviews platform under the number CRD42021214632. The PECOS research question was: Do adults and elderly individuals with diabetes mellitus, either type 1 or 2, have significant alterations in the salivary biochemical parameters than those without diabetes mellitus?

Search strategies

The following databases were searched: PubMed / MEDLINE, Embase, LILACS, Web of Science, Scopus, Science Direct, Cochrane, Livivo, as well as the grey literature Proquest for dissertations and theses, Google Scholar, OpenGrey, and search in the references lists. To assess the salivary profile in adults with type 1 and 2 DM, the main terms added in the search strategy was "adult, aged, elderly or middle aged", "Diabetes Mellitus, Diabetes Mellitus Type 1, Insulin-Dependent, Diabetes Mellitus Type 2, glycemic index, Glycemic Control, Blood Glucose, glucose control, Blood Glucose Monitoring, blood glucose control or blood sugar "and" Saliva, salivary profile, salivary parameters, salivary pH, buffer effect, buffer capacity, salivary flow, salivary calcium, salivary phosphorus, salivary urea, salivas, buffers or buffers. MESH terms were also included. The complete search strategy can be found at the appendix 1. The search date for all databases was 19 March 2021.

Inclusion criteria

Observational and clinical studies were included, with no time or language limitations, where adults and elderly (>30 years) with and without type 1 or 2 DM were the study populations. Studies with two groups (patients with DM1 or DM2 and healthy controls) were included, which evaluated the salivary profile, including salivary flow, pH, buffer capacity, urea, calcium, phosphate, or more than one of these variables in the same study. Studies should also include test

individuals with at least 6 months of diagnosis of DM previous to the salivary analysis.

Exclusion criteria

Studies were excluded if: 1) the population included edentulous individuals with Sjögren's Syndrome, who underwent radiotherapy, with severe systemic complications, transplanted or with systemic conditions that may influence the physiology of the salivary gland (eg hyper or hypothyroidism, chemotherapy in the last 3 months), pregnant women or lactating women and smokers; 2) studies without a control group (without DM or type 1 DM), animal or *in vitro* studies, and full-text articles not available (Reviews, book chapters, opinions, letters, conference abstracts, study protocols, case reports, case series); 3) studies that did not report the mean or median of the salivary profile, that described the salivary profile in groups under 30 years of age, or that did not specify the age range of the individuals included, or did not present relevant data.

The selection of the studies

Two independent reviewers researched the studies and selected the titles and abstracts for each study based on the eligibility criteria. Afterward, the same reviewers independently assessed the full text, confirming the eligibility. The discrepancies were resolved with the involvement of a third reviewer. The Rayyan tool was used in the both phases of study selection.

Data extraction

The data obtained for each study included the first author, year of publication, country, the sample size of patients and controls, proportions of male patients and controls, mean age of patients and controls, salivary collection methods, salivary flow rates, pH, buffer capacity, levels urea, calcium and/or phosphate, and main conclusions. In the case of intervention studies, the baseline data was extracted.

Methodological quality and risk of bias in individual studies

The same reviewers independently assessed the methodological quality of individual studies, using the JBI Critical Appraisal Checklist for Analytical

Cross-Sectional Studies (10). For clinical and longitudinal studies, the same tool was applied because only the data from the baseline was considered. Due to the design of included articles, besides all eight questions of the adopted appraisal tool are considered important, two of them were considered highly critical domains to this systematic review, including “Was the exposure measured in a valid and reliable way (considering standard diagnostic of DM)?” and “Were objective, standard criteria used for measurement of the condition (considering the criteria used to define salivary parameters)?”. A decision of **excluding** the studies with “no” answers for at least one of these domains was implemented. Another two were considered critical. These included: “Were the criteria for inclusion in the sample clearly defined?” and “Were the study subjects and the setting described in detail (description of the sample, such as age, gender, DMFT, etc.)?”. Criteria related to the salivary parameters were considered non-critical (criteria numbers 5-8).

Criteria adopted to this systematic review for considering a low methodological quality: two “no” or one “no” and one “unclear” or two “unclear” in critical domains, or two “unclear” and one or more “no” in non-critical domains. High methodological quality was considered when an article got a maximum one “no” answer or two “unclear” answers in non-critical domains. Papers with two “no” in highly critical domains were excluded. Decision on critical and non-critical domains and classification system was discussed with research team before the application of the instrument, as described at JBI Reviewer’s Manual (10).

Quantitative synthesis

A rigorous synthesis of the data and summaries of the results was carried out for each study, calculating the standardized mean differences and generating seven metanalyses. Due to the great variability of methods in the literature, the estimates of individual effects of each study were combined using the meta-analytical model of random effects and presented as differences in weighted means and 95% confidence intervals. The heterogeneity between the studies was estimated by the Cochran Q test and the inconsistency was tested by the I^2 statistic. When necessary, the characteristics of the study considered to be potential sources of heterogeneity were analyzed using sensitivity analysis or subgroup analysis, or both.

2.4 RESULTS

Studies selection and risk of bias

A total of 5817 studies were found by searching the databases. After excluding the duplicates, 1275 studies were recorded for screening, from which 1212 studies were excluded based on the PECOS question for defining the inclusion criteria. Out of the remaining 62 studies, 40 other studies were excluded based on the exclusion criteria [Figure 1, appendix 2].

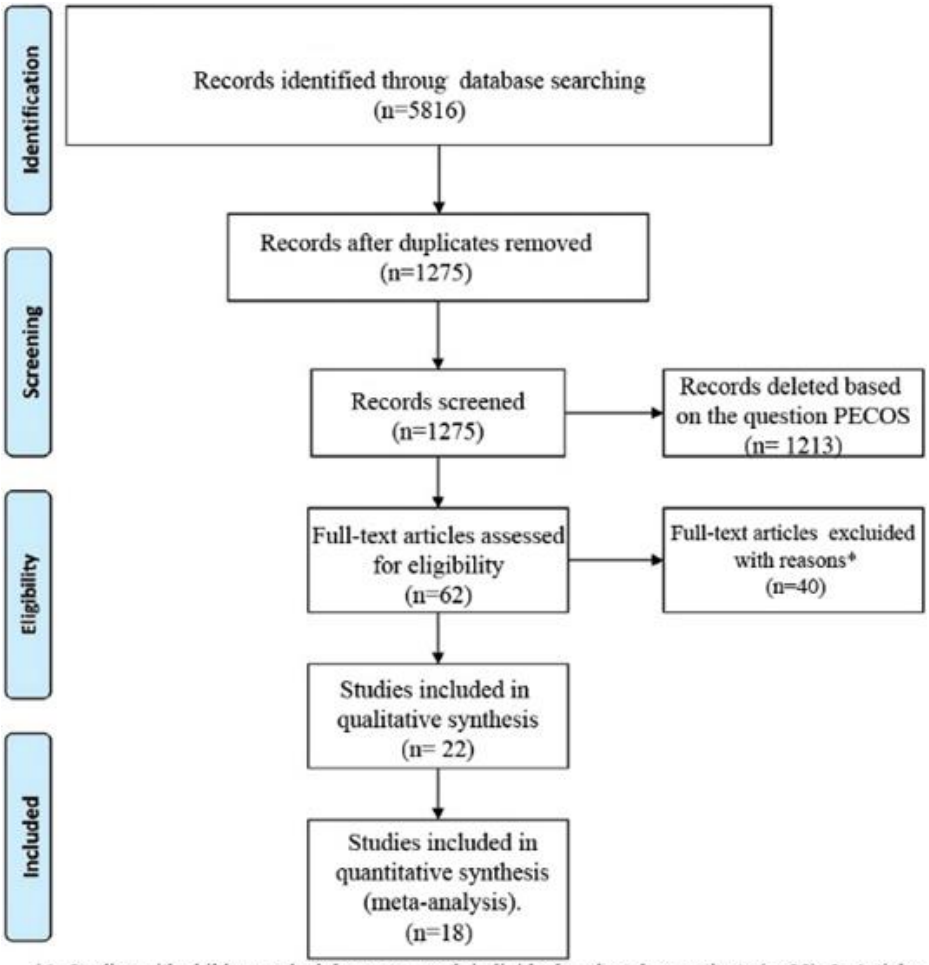
Table 1 shows the baseline characteristics of the 22 included studies in the qualitative synthesis. The studies were published from 1998 to 2021, and geographically well distributed (8 studies were reported in Brazil, one in the USA, two in Iran, five in India, one in Nigeria, one in Italy, one in Serbia, one in Finland, one in Poland, and one in Greece). There were 1202 and 946 individuals with DM (1077 with T1D, 34 with T2D and 91 did not specify type of DM) and healthy controls across all studies, respectively. Twelve studies measured the unstimulated flow, nine studies measured the stimulated flow, nine studies measured the salivary pH, four studies measured the buffering capacity, four studies measured the salivary calcium, four studies measured the salivary phosphorus and three studies measured the salivary urea.

Although the adopted exclusion criteria were not the same for all studies, most studies adopted similar exclusion criteria regarding the population sample for DM and control groups. In the salivary collection, most studies followed a fasting period of at least 2 hours before collection, and one study reported at least 90 minutes of fasting; in five studies this information was not reported; Most studies performed saliva collections in the morning, but three studies did not report at what time of day the collections were performed. In saliva collection to assess stimulated flow, a greater number of studies used paraffin for mechanical stimulation, followed by stimulation with latex rubber, and only one study used sublingual citric acid solution to obtain flow. Participants' information such as type of DM, duration of diabetes, concomitant medications, or systemic disorders was also not present in all studies.

Of the nine studies that evaluated pH, four used pH strips, and five used digital meters. In the analysis of salivary components, of the four studies that evaluated calcium, two used digital meters, and two used techniques using

atomic absorption spectrometers. In the phosphorus evaluation, two studies used digital meters, and one study used techniques using atomic absorption spectrometers. The four studies that measured salivary urea used techniques using atomic absorption spectrometers. All studies provide the results of the analyzes in the mean of difference.

Methodological quality assessment of selected studies was determined and detailed at appendix 3. The application of the defined criteria resulted in eight articles with a low quality, one with moderate and 13 with high quality. Criteria that impacted more at the methodological quality assessment were related to the definition of criteria to deal with confounding factors, such as age, salivary collection period, fasting before collections and glycemic control.



*1- Studies with children and adolescents, youth individuals, edentulous patients (n=28). 2- Articles with full text not available (Reviews, Book chapters, opinions,letters, conference abstracts, study protocols, case reports, case series or duplicate data (n=2); 3-Numeric data on either the sample or salivary parameters not specifically described.(n=9) 4- Studies measuring salivary parameters other than biochemical, such as protein and immunological markers (n=1).

Figure 1: Flowchart of the study

Table 1- Characteristics of the included studies

Author/ year	Gender (%)	Sample		Status DM (controlled or uncontrolled)	Type of DM	M (mean ±SD/range) year old	Age C (mean ±SD/range) year old
		M(n)	C(n)				
Chávez M. et al. 2001 USA	48,7% M 51,3% F	24	15	Controlled and uncontrolled	II	54-90+ yo	54-90 + yo
Balan P. et al. 2015 India	65% M 35% F	60	30	controlled and uncontrolled	II	48,33 ±8,46247 yo : 47,833 ±7,022 yo	48,20 ±7,131 yo
Rahiotis C. et al. 2021 Greece	54% M 46% F	23	18	uncontrolled and uncontrolled	II	C:64,3 ±10,32 yo C: 61,2 ±12,2 yo	62 ±9,63 yo
Oyetola E. et al. 2019 Nigeria	46% M 54% F	100	100	NA	NA	54.13+12.05 yo	55.48+12.4 yo
Vasconcelos A. et al. 2010 Brazil	50% M 50% F	40	40	NA	II	35-78 yo	50,2 ±12,3 yo
Bernardi L. et al. 2018 Brazil	5% M 95% F	10	10	Controlled	NA	52,7 ±10,17 yo	47,6 ±7,07 yo
Preejitha, V et al. 2019 India	NA	25	25	NA	II	30-60 + yo	30-60 + yo
Masoumeh S. et al. 2013 Iran	34% M 66% F	25	25	Controlled	II	30–45 + yo	30-45 + yo
Prathibha K. et al. 2013 India	50% M 50% F	30	30	NA	II	40 –55 + yo	40-65 + yo
Pendyala G. et al. 2013 India	50% M 50% F	60	60	NA	II	40 –65 + yo	40 –65 + yo

DM: diabetics; C = nondiabetics; F= Female; M= Male; WC= controlled Diabetes Mellitus PC = poorly controlled Diabetes Mellitus; NR, not reported

Table 1 (continued)

Kogawa M. et al. 2016 Brazil	50% M 50% F	63 37 WC: 31 TC: 32	Controlled and uncontrolled	II	34 -70 + yo	34 -70 + yo
Malickaa B. et al. 2014 Poland	50% M 50% F	Type I: 34 30 Type II: 59 33	Controlled and uncontrolled	I and II	Type I: 37,5 yo type II: 65 yo	Type I: 37 yo Type II: 63,7 yo
AmbiK U. et al. 2018 Brazil	32% M 68% F	24 24	NA	II	40-64 + yo	40-54 + yo
Meurman J. et al. 1998 Finland	52 % M 48% F	45 77	8.2 ±1.9 in men 9.2 ±2.2 in women	NA	59-79 +yo	59-77 + yo
Soares M. et al. 2004 Brazil	26% M 74% F	50 50	NA	II	68±6,6 yo	66±5,6 yo
Lopes D. et al.2017 Brazil	31,6% M 68,4% F	120 60 (60insulin-dependent AND 60 Non-insulin-dependent)	NA	II	72,26 ±6,53 yo	72,26 ±6,53 yo
Carramolino E. et al. 2018 Italy	40.4% M 59,6% F	47 46	NA	II	61.02 ±6.01 yo	59.43 ±5.20 yo
Kogawa M. et al. 2015 Brazil	39% M 61% F	72 38	controlled	II	WC: 57,50 ± yo PC: 56,50 ± yo	51+ yo
Mozaffari R. et al. 2019 Iran	44% M 56% F	100 100	serum glicose 161±59.7 (71-372); HbA1c 8.6±1.6 (6-13.2)	II	32-68 ±yo	55,0±9.3 + yo
MAHESWARI T. et al. 2016 India	NA	20 20	NA	NA	30-76 ± yo	30-76 + yo
Bernardi M. et al. 2007 Brazil	40,2% M 59,8% F	PC :63 18 WC:19	controlled and uncontrolled	II	54,3 ±10,1 yo	57,7 ±15,6 yo
Djuki L. et al. 2015 Serbia	38,7% M 61,3% F	91 60	NA	II	64,15 ±6,94 yo	57,47 ±5,75 yo

DM: diabetics; C = nondiabetics; F= Female; M= Male; WC= controlled Diabetes Mellitus PC = poorly controlled Diabetes Mellitus; NR, not reported

Tabela 2- Characteristics of salivary parametes within the included studies

Author/ year/ Country	Stimulated salivary flow (ml/min)		Unstimulated salivary flow (ml/min)		Buffering Capacity		pH		Ca DM (mg/dL)		P (mg/dL)		Urea (mg/dL)	
	DM	C	DM	C	DM	C	DM	C	DM	C	DM	C	DM	C
Chávez M. et al. 2001 USA	NA		WC: 0,14 ±0.13 PC: 0,18 ±0,25	0,26 ±0,29	NA		NA		NA		NA		M: 10,4±5,6; F: 13,5±6,67	M:0,861±0,38 F:0,76±0,40
Balan P. et al. 2015 India	NA		NA		NA		0,900.	0,033	NA		NA		NA	
Rahiotis C. et al. 2021 Greece	4,87 ± 1,70	6,5 ± 2,44	NA		5,12 ± 2,35	8,15 ± 2,47	7,18 ± 0,45	7,57 ± 0,31	NA		NA		NA	
Oyetola E. et al. 2019 Nigeria	NA		M: 0,32±0,13 F: 0,31±0,1	M:0.68±0.320 F:0,68±0.36	NA		11±0.70	7.66±0.65	NA		NA		10,4±5,6	0,861±0,38
Vasconcelos A. et al. 2010 Brazil	0,63±0,43	1,20±0,70	0,21± 0,16	0,33±0,20	NA		NA		NA		NA		NA	
Bernardi L. et al. 2018 Brazil	0,47±0,11	1,4±0,38	NA		NA		NA		NA		NA		NA	
Preejitha, V et al. 2019 India	NA		NA		NA		6,4±0,66	7,42±0,50	NA		NA		NA	
Masoumeh S. et al. 2013 Iran	NA		0,18±0,14	0,30±0,12	NA		NA		9,22±2,32	9,47 ±0,76	12,71±4,62	11±4,85	29,05±3,81	19,7 ±1,4
Prathibha K. et al. 2013 India	NA		0,46±0,02	0,67 ± 0,07	NA		6,69 ± 0,35	7,09 ± 0,29	4,22 ± 0,12	6,39 ± 0,5	NA		NA	
Kogawa M. et al. 2016 Brazil	NA		NA		WC: 3.47±1.23 PC: 4.0±1.22;	4.29±1.33	NA		NA		NA		NA	
Malickaa B. et al. 2014 Poland	NA		T1D:0,38 ±0,19; T2D: 0,36 ±0,21;	0.5 ±0,2 0.45 ± 0,25	NA		NA		NA		NA		NA	
AmbiK U. et al. 2018 Brazil	NA		NA		NA		NA		Diabetic with caries 6,70; Non-diabetic with caries 6,97 Diabetic without caries 6.64; Non-diabetic without caries 7.72		Diabetic with caries 13,45; Non-diabetic with caries 9,48 Diabetic without caries 12.52 Non-diabetic without caries 12.3		NA	
Meurman J. et al. 1998 Finland	1,2 ± 1,4	1.2 ± 0,8	0,3 ± 0.3	0,3 ±0,2	7,8%	13,9%	NA		NA		NA		5,3 ± 1,7	4,8 ± 3,1

DM: diabetics; C = nondiabetics; F= Female; M= Male; WC= controlled Diabetes Mellitus; PC = poorly controlled Diabetes Mellitus; T1D= type 1 Diabetes; T2D= type 2 Diabetes; P: Phosphorus; Ca: Calcium; NR, not reported; NA, not applicable.

Tabela 2 (continued)

Soares M. et al. 2004 Brazil	1,58±0,84	1,91±0,98	0,27±0,26	0,32±0,27	NA		6,56±0,79	6,56 ± 0,81	NA		NA	NA
Lopes D. et al.2017 Brazil	F: 0,47±0,4 M: 0,63±0,44	0,54±0,42	NA		NA		NA		NA		NA	NA
Carramolino E. et al. 2018 Italy	0,88±0,63	1,04±0,64	WC: 0,88 PC: 0,72	1,07	NA		NA		NA		NA	NA
Mozaffari R. et al. 2019 Iran	NA		NA		NA		6,9±0,25	7,08±0.25	1,80±2,29	2,15±1,67	12,83±7,84	19,59±12,62	34,16±20,71	29,28±13,17
MAHESWARI T. et al. 2016 India	NA		NA		NA		4.3±0.08	7.7±0.20			NA	NA	NA	
Bernardi M. et al. 2007 Brazil	WC: 0,81 ± 0,47 PC: 0,65 ± 0,62	1,95 ± 0,73	NA		WC: 4.81 ± 1.21 PC: 4.34 ± 1.58	4,45 ± 1,45	PC: 6,8 ± 0,9 WC: 6,7 ± 1,3	6,7 ± 1,8			NA	NA	NA	
Djuki L. et al. 2015 Serbia	NA		0,28	0,34	NA		NA				NA	NA	NA	

DM: diabetics; C = nondiabetics; F= Female; M= Male; WC= controlled Diabetes Mellitus; PC = poorly controlled Diabetes Mellitus; T1D= type 1 Diabetes; T2D= type 2 Diabetes; P: Phosphorus; Ca: Calcium; NR, not reported; NA, not applicable.

Quantitative synthesis

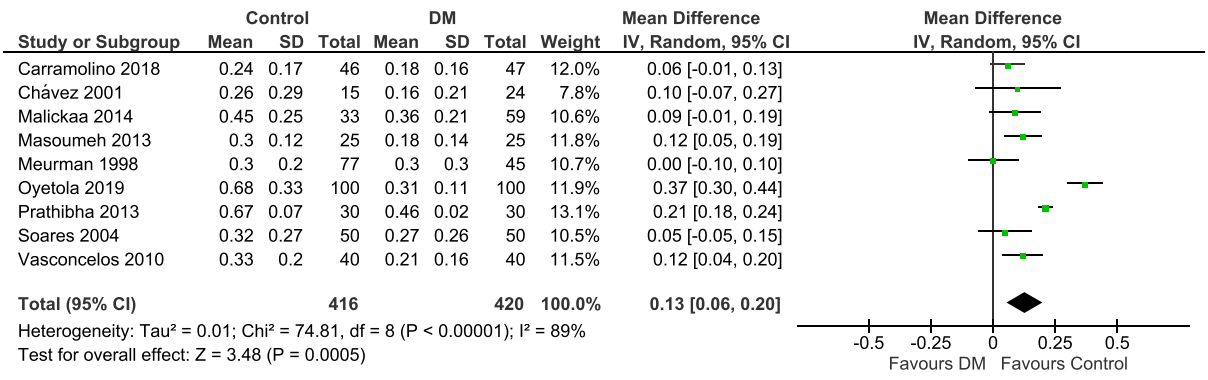
Meta-analyses comparing averages of the salivary parameters between DM and normoglycemic controls are presented at figures 2 to 6. The unstimulated salivary flow was 0.13 ml/min (Confidence Interval-CI= 0.06-0.2) higher for control individuals than DM ones (figure 2A), while the stimulated salivary flow was 0.5 ml/min (CI= 0.15-0.84) higher in controls than DM (figure 2B).

Although a great sample size was reached, comprised by a total of 430 cases and 361 controls from 8 studies, the metanalysis for the salivary pH could not show statistically significant differences between groups, although the pH tends to be higher for control individuals (0.73 points higher, figure 3). Regarding the buffering capacity (figure 4), it was possible to divide the analysis into two subgroups according to the level of glycemic control of the test individuals described in the included studies: poorly or well controlled DM. No significant differences were found, regardless of DM control level.

Figure 4 depict the comparison of the salivary calcium (A) and phosphorus (B) concentrations in individuals with DM and controls. Although the average of the salivary calcium concentration was higher for control individuals (0.96 mg/dL higher) non- significant statistic difference was observed. The opposite pattern was observed for the salivary phosphorus concentration (slightly higher for DM subjects, 2.5 mg/dL higher, but non-significant statistic difference). Interestingly, the salivary urea was significantly higher for DM individuals (average 6.67 mg/dL higher; CI= 0.08-13.25).

Figure 2. Forest plot comparing the salivary flow in individuals with Diabetes Mellitus (DM) and normoglycemic controls. A= Unstimulated salivary flow; B= Stimulated salivary flow.

A



B

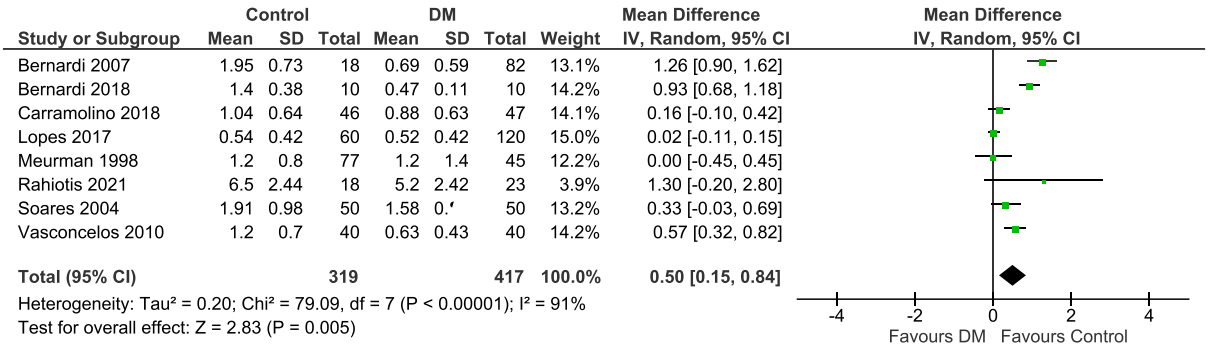


Figure 3. Forest plot comparing the salivary pH in individuals with Diabetes Mellitus (DM) and normoglycemic controls.

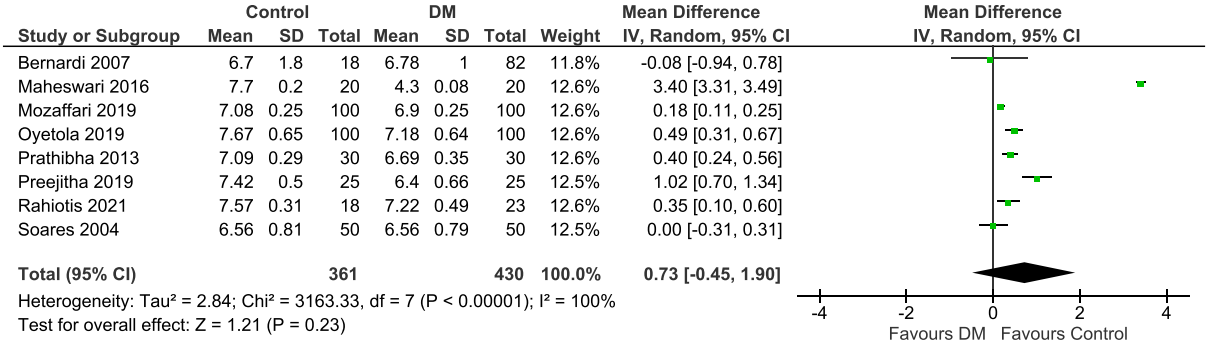


Figure 4. Forest plot comparing the salivary buffering capacity in individuals with Diabetes Mellitus (DM) and normoglycemic controls, divided into two subgroups: poorly controlled DM and well controlled DM.

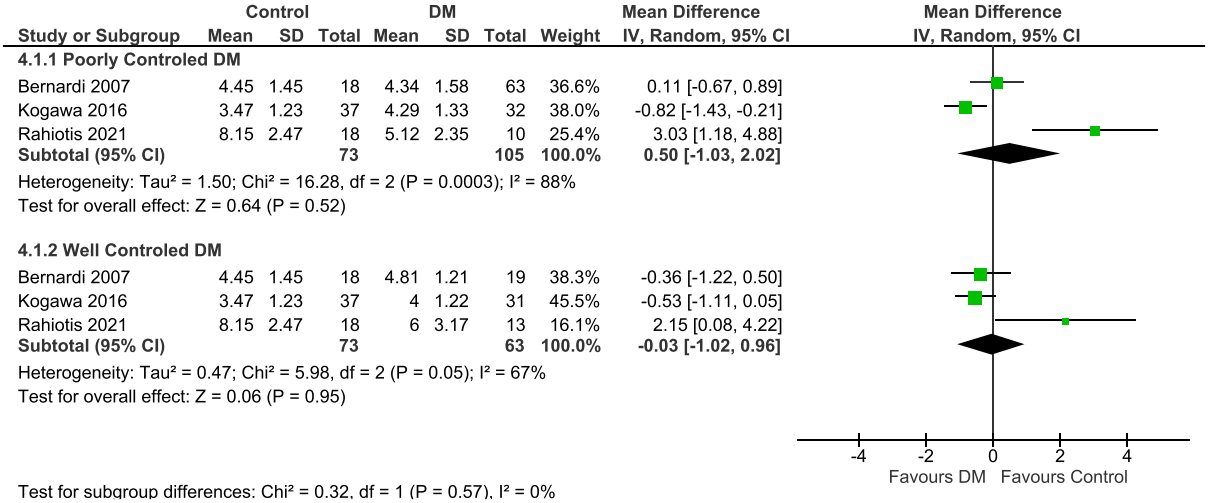
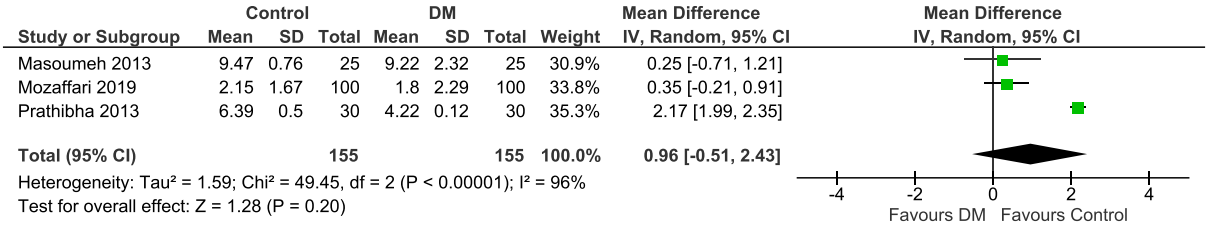


Figura 5. Forest plot comparing the calcium (A) and phosphorus (B) in individuals with Diabetes Mellitus (DM) and normoglycemic controls.

A



B

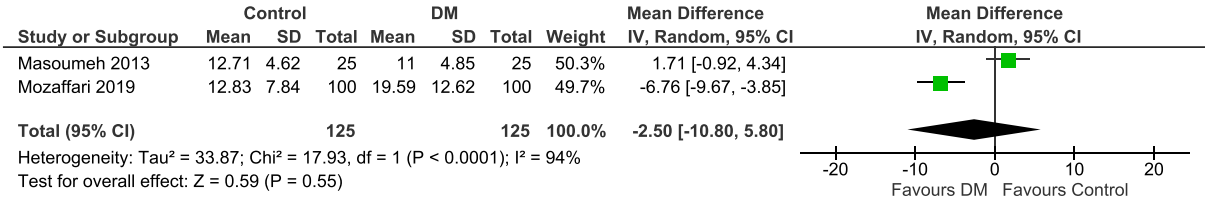
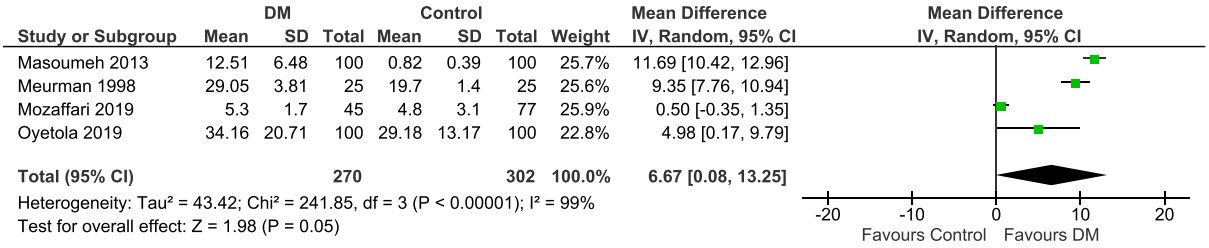


Figura 6. Forest plot comparing the urea in individuals with Diabetes Mellitus (DM) and normoglycemic controls.



2.5 DISCUSSION

There are several studies in the literature evaluating salivary parameters, however, these studies cover different age groups. Age also has an influence on changes in human physiology, and our objective was to compare salivary parameters only in adults, exposed or not to DM, to avoid confounding bias. Significant alterations were observed in the DM saliva, which can be an indication for the importance of diagnosing and monitoring those altered parameters such as salivary flow and urea concentration in all DM individuals to maintain their oral health. Although most studies demonstrate that pH in DM is lower than normoglycemic controls, no significant differences were found here, as well as for buffer capacity, salivary calcium and phosphorus concentrations (7,8).

In addition to systemic changes, prolonged hyperglycemia can alter the function of the salivary glands that result in changes in the composition and volume of secreted saliva, due to polyuria or changes in the basement membrane of the salivary glands, medication effects, and absence of parasympathetic stimulation of the salivary gland (11,12). In the analysis of the included studies, individuals with DM had a significantly lower unstimulated salivary flow rate than in the control group in ten studies (3,11,13–19), and only two did not show significant results, although the control groups had a greater mean difference in DM in these studies (20,21). From those studies, it was possible to meta-analyse 9 which confirmed a difference in the average salivary flow between DM and normoglycemic controls. Regarding stimulated salivary flow, 5 studies showed a significant reduction in the flow in DM (14,22–25), and another four did not report significance (18,20,21,26). However, three out of them not clearly detailed the salivary collection methods, such as collection period and fasting. Possible causes for this contradiction may be associated with differences in saliva collection methods (stimulated or non-stimulated), at the time of collection, and in the conditions and position of the patient at the time of collection. Differences between salivary flow at rest and stimulated, can be credited to the production of afferent signaling that induces an increase in flow in the affected population after mechanical salivary stimulation (chewing). This explains the importance of collecting the salivary flow at rest, to prevent the use of stimuli in the collection

from camouflaging the actual performance in salivary production of these patients. Standardizing assessment conditions, such as collection period and duration, are also important to avoid possible assessment biases.

In hyperglycemia, a deficiency in insulin, which is a fundamental hormone for the regulation of blood sugar levels, results in an increase in other hormones such as glucagon and cortisol, that induce areas of the body that usually degrade sugars through the action of insulin, begin to degrade fats. It is this “fat-burning” to produce energy, due to the unavailability of sugars, which ends up producing ketone bodies that accumulate in the blood, and make the blood more acidic, with a drop in serum ph (27). At the oral cavity, changes in the salivary pH favors the growth of periodontal pathogens or the aciduric bacteria, creating an inhospitable environment for protective oral bacteria, which can lead to the aggravation of periodontal diseases and development of carious lesions, which further reduces the salivary pH, leading to a repetitive cycle (28). Regarding pH, six studies showed significant low pH rates in DM when compared to controls, and only 3 did not find significance. The difference can be attributed to the different evaluation methods, as five studies used electronic meters to assess pH, the most sensitive method for measuring pH, and four used pH strips. In the evaluation of the buffering capacity, two studies did not find a significant difference, and in two significant but conflicting results were found: in one study the DM group had greater buffering capacity (22), in the other, DM had lower buffering capacity (25).

Disagreements may be due to the use of colorimetric methods and electrometric methods to determine buffer capacity. However, neither pH or buffering capacity were significantly different between DM and controls, even with the higher number of individuals that were evaluated in the present analysis (figures 3 and 4).

The presence of calcium and phosphate in saliva in high concentrations is a beneficial indicator of a person's oral health. Salivary calcium plays a favorable role in enamel remineralization (29). These components in saliva are essential to maintain a balance in the relationship between mineralization and demineralization of enamel (30) and can be a potential biomarker of health status. DM patients may have their salivary calcium levels affected, because insulin secretion is a calcium-dependent process, and consequently reduce serum

calcium levels, and this reduction in serum reflects in on salivary rules (30). In addition, another study found that an increase in blood glucose increases urinary calcium and phosphorus excretion, which is proportional to the degree of glycosuria (31). Regarding salivary calcium, all studies evaluated presented a tendency of lower average of calcium levels in DM compared to the group without DM, however, significant differences between the groups were not observed. In the three studies that evaluated salivary phosphorus, there were conflicting results: one study showed significantly lower levels of phosphorus in DM in another, phosphorus rates in DM were significantly higher than those found in controls, and one study found no significant difference between groups. Different from the other parameters evaluated here, it seems that the sample size is low and that more studies are needed to test the hypothesis of Ca and P at the saliva of DM.

Diabetic nephropathy is a complication caused by DM in the renal microvasculature. Individuals with DM have a higher glomerular filtration rate or hyperfiltration, mediated by greater relaxation of the afferent arterioles compared to the efferent arterioles. This in turn leads to increased blood flow through the glomerular capillary, increasing pressure. When these conditions are maintained over time, they produce glomerular hypertrophy and an increase in the surface of the glomerular capillary. This leads to hemodynamic changes that contribute to the development and/or progression of the disease (32,33). Increased plasma urea is a basic sign of kidney problems, and this could reflect in the urea concentration at saliva. Consistent with the scientific literature, salivary urea is significantly higher in diabetic patients (34,35) (figure 6). All included studies showed higher averages of urea in DM when compared to controls (13,19,20,36), and in three (13,19,36) these results were significant. Knowing that nephropathy is not uncommon in patients with DM, salivary urea seems to be a promising future marker in the monitoring of patients with nephropathy. More studies are necessary correlating serum urea and salivary urea, to verify if salivary urea can be used as a biomarker to indicate systemic disorders, such as nephropathy. Also, little is known about the impact or relationship that these high levels of salivary urea may have on the oral health of individuals with T2D, requiring further investigation in the future.

Within the limitations of this study, advanced age plays a vital role in changing salivary parameters in diabetics, being a confounding bias. Also, only few studies did not report the glycemic status of the sample, nor the type of DM, making these comparative subanalyses impossible for this group. Furthermore, new cohort studies are necessary to increase the strength of the evidence.

2.6 CONCLUSIONS

DM leads to a remarkable change in saliva parameters, especially in salivary flow and in the urea levels compared to normoglycemic individuals. In this context, the routine analysis of salivary parameters might be performed in the daily clinical practice in order to improve the diagnosis of the systemic and buccal diabetes complications and better perform the risk assessment of dental caries.

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3. CAPÍTULO 3- SALIVARY PARAMETERS ASSOCIATED WITH DENTAL CARIES IN INDIVIDUALS WITH TYPE 2 DIABETES MELLITUS: A CROSS-SECTIONAL STUDY

3.1 ABSTRACT

Introduction: it has been suggested that diabetic patients are in increased risk for dental caries and the biological reasonability has been credited to altered salivary parameters, such as salivary pH, salivary flow, and buffering capacity. However, the occurrence of dental caries in type 2 diabetes (T2D) is not totally understood so far, especially in the elderly population.

Objectives: This cross-sectional study aimed to compare salivary parameters and the occurrence of caries in patients with type 2 diabetes mellitus (T2D) and patients without T2D and to evaluate the impact of inadequate glycemic control in saliva and the prevalence of caries.

Methods: 54 individuals participated in the study, of which 35 belonged to the systemically healthy control group and 19 to the T2D group. Subjects were then classified as controlled or uncontrolled (HbA1c≥6.5 and/or FBG>100). The examined clinical parameters were: active coronal caries and root caries (decayed surfaces only, prevalence and extent), salivary pH, salivary flow, buffering capacity, and salivary amylase.

Results: T2D individuals had a greater number of root caries lesions than individuals without DM (p = 0.011). The average of triglycerides was higher in individuals without active coronal caries (p = 0.007), although HDL rates showed an opposite trend (p = 0.05). Averages of unstimulated salivary flow were lower in individuals with uncontrolled DM (p = 0.009), as well as the salivary pH (p = 0.03). In the regression models, diabetics are 2.25 times more likely to have increased number of surfaces with root caries (p=0.046) after adjustment for age and unstimulated salivary flow.

Conclusions: Salivary parameters such as the reduction in unstimulated salivary flow do not explain by themselves the greater number of root caries

lesions in patients with T2D. More factors are involved to explain the increased risk of root caries in these individuals and this association should be evaluated longitudinally.

3.2 INTRODUCTION

Oral manifestations are common and well-studied in individuals with Diabetes Mellitus (DM). The condition of hyperglycemia induces inflammatory responses, which contributes to the systemic degradation of connective tissues, including periodontal structures, and a decrease in the oral pH (1). Other manifestations of DM might be related to the changes in the salivary composition and functions (2,3). There is a consensus in the literature that salivary glucose levels is correlated to the serum glucose levels in individuals with DM (2). Altered salivary flow and composition observed in individuals with uncontrolled DM can, theoretically, compromise its protective function and predispose them to dental caries (4). The plausibility is regarding the understanding of saliva as a complex biological fluid that contains several biomolecules that collaborate for the ionic balance of hydroxyapatite in dental tissues, remineralization, antimicrobial function, regulation of oral pH, and for its buffering capacity. When the salivary flow decreases, there may be an increase in the concentration of mucin and glucose, which results in the change of the oral environment by favoring the colonization of acidogenic bacteria that can increase the cariogenic capacity (5). Examples of changes in salivary enzymes that can also impact the oral health of DM individuals include the amilase (6). Considering that fasting DM individuals have high levels of salivary amylase (3), which would favor a great variety of species in the oral microbiome (6), becomes another indication that the high levels of salivary glucose in these individuals is the salivary alteration correlated to the low salivary pH, and consequently, to the imbalance of the oral microbiome and greater exposure the risk of caries (5).

It has been shown that the prevalence and severity of dental caries among children and adolescents with DM is higher than in the general population(7). In

addition, a systematic review and metanalysis developed by our research group found an increased chance of adults with DM type 2 (T2D) having coronal and root caries compared to individuals without DM (8). We also showed that individuals with uncontrolled glycemic levels were 3.8 times more likely to have caries than the controlled ones, as well as a higher DMFT (number of decayed, missing and filled teeth)(8). We concluded that the relationship between diabetes with dental caries, particularly among adults, has received less attention so far due to the lack of longitudinal studies. However, we believe that the caries detection criteria strongly influenced the results. The missing component of the DMFT (the most used criterion for caries detection in that review) might inflate estimates as it represents either the cumulative caries experience throughout life or even teeth extracted by other reasons not related to dental caries, such as periodontitis(8). This indicates the need for new studies considering the activity in caries detection to avoid the past caries experience influence.

Although both diseases (DM2 and caries) embrace the same causal factor, the unbalanced carbohydrate intake, other factors may be involved in the relationship between caries and DM. A hypothesis is the previously discussed qualitative and quantitative difference in the saliva of DM individuals, that should be influenced by its glycemic control levels (9,10).There is a lack of more consistent evidence and studies that confirm the role of changes in salivary flow and composition in adult individuals with T2D and their direct relationship with the likelihood of caries development. Thus, this study aimed to compare salivary parameters (salivary flow, pH, buffer capacity, and salivary amylase) of individuals, exposed or not to DM, with and without coronal and root caries activity.

3.3 METHODS

Ethics

This study was approved by the Ethics Committee of the School of Health Sciences at University of Brasilia (process No. 87962818.4.0000.0030), in accordance with the declaration of Helsinki. All patients signed a informed consent form and received basic periodontal treatment, as well as restorations and endodontic treatments when necessary. The study was funded by FAP-DF (process no. 16991.78.45532.26042017).

Study Design

The present study has a cross-sectional design, aiming to associate the impact of salivary changes with the prevalence and extent of active caries lesions, in individuals with T2D, associated or not with periodontitis. This report followed the STROBE checklist.

Sample

Individuals attended at the Diabetes dental clinic of the University Hospital of Brasilia were recruited from June 2018 to March 2020. The study was carried out on-demand from the hospital. The sample consisted in adults, of both sexes. Initially, the following information was collected during anamnesis: sociodemographic data (age and sex), types of medications used, oral hygiene habits, and diet.

Eligibility criteria

Patients with T2D, without periodontitis (DM) and with periodontitis (DMP: at least two teeth with interproximal insertion loss ≥ 3mm), aged over 30 years, regardless of the level of metabolic control, were included. Control individuals with periodontitis and systemically healthy (group P), and systemic and periodontal healthy individuals were also included (group C). DM status was established by a reported medical diagnosis of T2D and by the use of either insulin or other oral hypoglycemic medications.

Individuals with type 1 DM were excluded, as well as the ones with complications and severe systemic comorbidities; transplanted patients; patients with a positive history of epilepsy; with the presence of systemic conditions that may influence the physiology of the salivary gland, such as hypothyroidism, and neck regions, radiotherapy or chemotherapy treatment that preceded 3 months. In addition, patients with mucosal lesions, pregnant, consumers of alcohol, and other illicit drugs were excluded.

Variables and measures

Assessment of Health condition and glycemic control

Participants were tested regarding their fasting blood glucose (FBG) levels and the glycated hemoglobin (HbA1c) within three months before salivary collection (Sabin laboratories, Brasilia, Brazil). Individuals were then classified as controlled or uncontrolled (HbA1c≥6.5 and/or FBG>100). The lipid profile was also determined at the same exam.

Assessment of oral condition

Periodontal clinical examinations were performed by previously trained examiners, with the following clinical parameters being observed: Plaque Index (PI), Bleeding on Probing (BP), Probing Depth (PD), Gingival Recession (GR), and Insertion Level (IL), mobility and degree of furcation involvement, recorded using a Williams-type periodontal probe (Hu-Friedy MFG. Co. Inc., Chicago, IL, USA). The number of remaining teeth was counted by visual inspection. Complementary panoramic X-ray examinations were also performed (a complete periapical series of each patient was also performed when clinically indicated). The data was combined and patients classified according to the new classification of periodontal diseases (11) and categorized as with or without periodontitis.

To assess coronal and root caries, the presence of both cavitated and non-cavitated lesions was recorded, including classification of the activity of the lesions (Nyvad, 1999). Prior to the examination, professional removal of dental biofilm was performed. The examination was performed under artificial light, in a

supine position, using clinical mirrors, WHO probes, and tooth air-dried and isolated with cotton rolls. Active non-cavitated were the ones with characteristics of opacity with a dullwhitish surface, while the inactives non-cavitated were shiny with different degrees of brownish discoloration. The cavitated lesions were active when had a localized surface destruction with active (dull-whitish enamel and soft dentin), and inactive cavitated were localized surface destruction with arrested characteristics (shiny and hard surfaces). For root caries, lesions were classified as active or inactive based on surface hardness. The number of surfaces with fixed prosthesis, root debris, non-carious lesions, sealant, and restorations were also recorded. For this assessment, the examiners were previously trained and calibrated. Only the examiners with inter and intra-examiners Kappa>0.7 performed dental examinations. Calibrations were performed every six months through a double examination of photographs, with a 7-days interval between them. Individuals were classified according to the prevalence of active coronal caries (at least one coronal surface affected with active caries, either cavitated or non-cavitated, respectively). Prevalence of root caries was individuals with at least one lesion of root caries (active or inactive). The extent of caries, was also calculated, considering the average number of surfaces with lesions per patient. These estimates included only the decayed surfaces (D-S), excluding the missing (M-S) and filled (F-S) components that can represent past caries experience.

Sialometry and saliva collection

The salivary flow measurement was performed in the morning (8-10 hours) to minimize the effect of circadian rhythms. The individuals were instructed to refrain from eating, drinking, and performing physical activity for at least two hours before the sialometry procedures.

Both unstimulated and stimulated saliva were performed. Unstimulated saliva was collected during 5 minutes at rest by passive drooling. The total stimulated saliva was collected for 5 minutes, by expectoration in a plastic container, after the patients chewed a device made of silicone during 1 minute. After collection, the saliva sample was aliquoted (500µL) and stored at -20ºC for

further laboratorial analysis. The parameters of salivary flow were measured by the volume of total saliva collected per minute (mL / min). The variables of salivary flow rate were analysed continuously.

Salivary viscoelasticity

The salivary viscoelasticity was measured during the transfer of unstimulated saliva to the microtube, being classified as serous, fluid, and viscous by a single examiner (a trained laboratory technician).

Salivary pH

The salivary pH analysis was performed with the aid of a pH indicator strip (MQuant® Merck, The pH Test 1-14.0). The strip was immersed in the collected saliva (unstimulated) and, between 30 seconds to 1 minute, and the color acquired by the tape reflected the pH result (indicated on the tape packaging itself).

Buffer capacity

The buffering capacity was measured from 1 ml of stimulated saliva from the collecting flask, added with 3 ml of 0.005M hydrochloric acid, and measured after 2 minutes of pH with an indicator strip. Values below pH 4.0 indicate low buffering capacity.

Salivary Amylase

The Amilase CNPG Liquiform Kit (Labtest, Lagoa Santa, Brazil) was used following the protocol recommended by the manufacturer, adapted to the type of sample. Briefly, 2 µL of the sample was added into 50 µL of the reagent, 50µL of substrate and 400µL of distilled water. A negative control with only 400 µL of distilled water was added to the experiment, as well as a standard containing only color reagent, substrate, and distilled water. Each sample was assessed in duplicate. All tubes were incubated in a water bath at 37ºC for 2 minutes initially, and after adding the sample in the respective tubes, they were again incubated in a water bath at 37ºC for 7 minutes and 30 seconds. The absorbance was evaluated in a spectrophotometer, in a microplate reader.

Statistical analysis

After confirming the non-parametric distribution of the data (Kolmogorov-Smirnov test), except for the variable triglycerides, differences between groups were tested using Mann-Whitney. Chi-square and Fisher exact tests were used to compare the distribution of the sample in the categorical variables. Spearman correlations were used to correlate the salivary flow with the blood glycemic levels. Unadjusted and adjusted binomial regression models were performed to observe the prediction of the number of coronal or root caries by the T2D (variables of adjustment: salivary pH, glycemic level control, unstimulated saliva). The level of significance was set at 5%. The data were analysed with SPSS version 26.0 and Prism 9 for MacOS v.9.0.2.

3.4 RESULTS

Fifty-four individuals met the selection criteria and participated in the study (16 male and 38 female), from which 30 belonged to the systemically healthy control group (C), 10 to the DM group, 5 to the P group, and 19 to the DMP group. The general characteristics of the sample (sociodemographic, health condition and salivary parameters) according to the prevalence of coronal and root caries are given in Table 1. The number of individuals with uncontrolled glycemic rates was 17. Almost all individuals with root caries were diabetic (p=0.011) and they were significantly older than the ones without root caries (age 46.93±12.61 without root caries and 60.31±8.39). The average of triglycerides was higher in individuals without active coronal caries when compared to the ones with coronal caries, although the HDL rates presented the opposite trend. Neither active coronal caries nor root caries were significantly associated with salivary parameters in this sample. Salivary amylase, buffer capacity, and salivary viscoelasticity were not significantly associated with caries extent, either coronal or root (Figure 2).

Regarding caries extent, individuals with DM had higher number of root caries lesions, but these rates were not affected by the glycemic control. Averages of unstimulated salivary flow were higher in individuals with uncontrolled DM, as well as the salivary pH (Table 2).

Stimulated and unstimulated salivary flow were not significantly correlated. Unstimulated saliva seems to better reflect the glycemic control, as there was a significant weak negative association with the HbA1c and the FBG, meaning that the unstimulated salivary flow significantly reduces with the increase of the glycemic indexes (Figure 3). No significant correlations were found between the stimulated saliva with FBG (r=0.012; 95% confidence interval-CI= -0.31-0.33) and HbA1c (r= -0.32; 95% CI= -0.60-0.03).

Table 3 displays the negative binomial regression models for root caries extent. Diabetics are 2.79 times likely to have an increased root caries extent when compared to non-diabetics. This number reduces to 2.71 and 2.25 times in models 1 (adjusted to controlled glycemic control and unstimulated salivary flow) and 2 (adjusted for age and unstimulated salivary flow), respectively. This

necessarily means that, even with the adjustment for the variables “unstimulated salivary flow” and “salivary pH”, diabetes still explains part of the increase in the number of root caries lesions. Glycemic control did not detract from the significance of diabetes itself either. However, age was very important in model 2 and explained a great part of the higher number of root caries lesions in DM group, but not removing the significance of the presence of diabetes. Although averages of salivary pH did not significantly differ regarding prevalence (table 1) or extent (table 2) of root caries, some association was observed at the unadjusted at the regression model, which was lost after adjusting the model for glycemic control and unstimulated salivary flow. Regression models for coronal caries were not significant (data not shown).

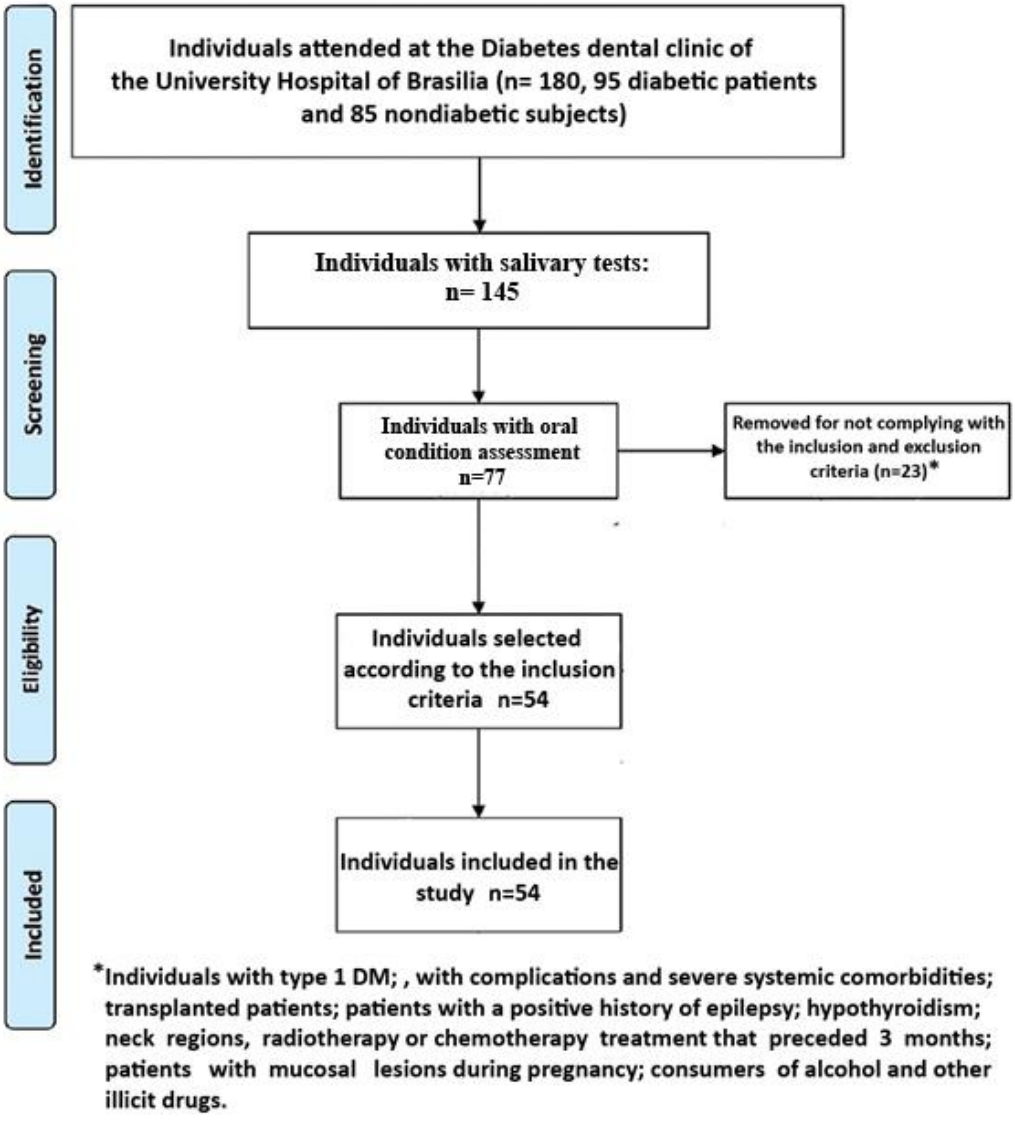


Figure 1. Flow diagram of the study

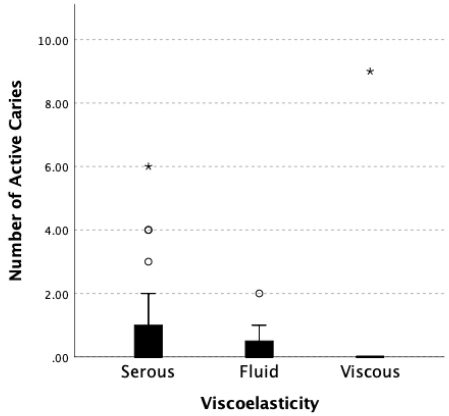
Table 1- Characteristics of the sample according to the prevalence of coronal and root caries

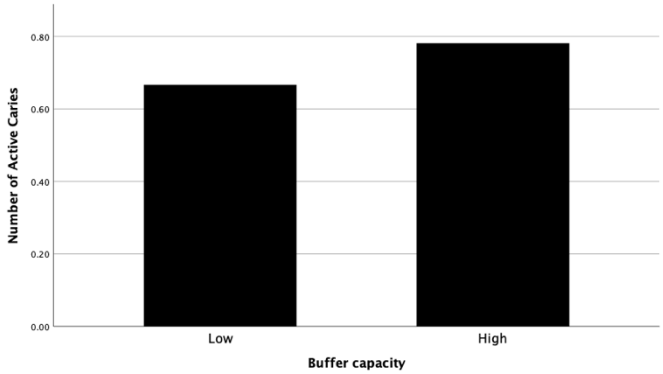
Variables		No active coronal caries (D-S)			Active coronal caries			p	No root caries			Root caries (D-S; including all active and inactive lesions)			p
		Average	N	SD	Average	N	SD		Average	N	SD	Average	N	SD	
Sociodemographic															
Gender	Female		27			11		0.337		27			11		0.174
	Male		13			3				14			2		
Age		50.05		13.22	50.43		12.87	0.96	46.93		12.61	60.31		8.39	**0.001**
Health condition															
Diabetes§	No		18			7		0.49		23			2		**0.01**
	Yes		22			7				18			11		
Periodontitis	No		16			8				15			9		
	Yes		24			6				24			6		
Total cholesterol†		193.25	20	43.05	196.89	9	44.22	0.66	194.95	20	46.44	193.11	9	35.25	0.69
Triglycerides†		162.70	20	93.98	102.33	9	45.87	**0.007**	138.35	20	60.03	156.44	9	130.76	0.66
Hdl† (mg/dl)		44.80	20	11.85	53.78	9	8.97	**0.05**	47.80	20	12.58	47.11	9	10.02	0.79
Ldl† (mg/dl)		117.46	18	40.92	251.78	9	393.50	0.46	120.76	18	43.83	245.18	9	395.27	0.82
Glycemic control*§	Yes		15			9		0.15		18			6		0.61
	No		14			3				13			4		
Salivary parameters															
Unstimulated salivary flow (ml/min)		0.31	40	0.27	0.24	14	0.23	0.31	0.31	41	0.27	0.22	13	0.22	0.16
Stimulated salivary flow (ml/min)		0.42	40	0.32	0.56	14	0.48	0.4	0.48	41	0.39	0.37	13	0.29	0.3

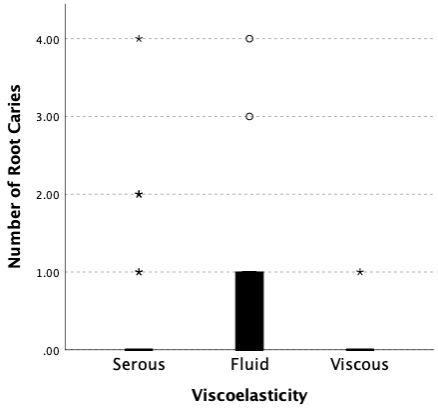
Salivary amilase		12919.8	40	5121.7	11672.4	14	5527.7	0.43	12203.5	41	5231.4	13835.8	13	5126.6	0.3
Salivary pH		7.05	40	0.59	6.92	14	0.82	0.56	7.02	41	0.61	7.00	13	0.82	0.91
Buffer capacity**	Low		15			6		0.58		14			7		
	High		24			8				27			5		
Salivary	1.00		20			8		0.74		22			6		0.59
Viscoelasticity	2.00		14			5				13			6		
	3.00		6			1				6			1		

*****Controlled=HBA1C>6.5 and/or FBG>100; †Mann-Whitney test; §Fisher exact test; **Chi-square test; D-S = non-cavitated or cavitated decayed surfaces.

Figure 2. Salivary characteristics and caries extent. A= Coronal caries and viscoelasticity; B= Root caries and viscoelasticity; C= Coronal caries and buffer capacity; D= Root caries and Buffer capacity (p>0.05, Fisher test).







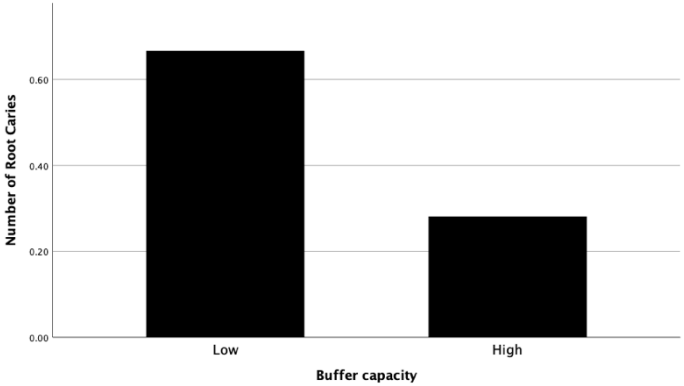


Table 2- Average of the salivary parameters, and coronal and root caries extent (only decayed surfaces – D-S) according to the diabetes prevalence and metabolic control.

	Without DM		With DM		P*	Controlled glycemic levels		Uncontrolled glycemic levels		P*
	Average	SD	Average	SD		Average	SD	Average	SD	
Number of surfaces with active coronal caries	1.04	2.24	0.45	0.91	0.74	0.54	0.83	0.94	2.33	0.55
Number of surfaces with root caries (active and inactive)	0.12	0.44	0.72	1.19	**0.01**	0.5	1.06	0.47	1.07	0.92
Unstimulated salivary flow (ml/min)	0.31	0.29	0.26	0.25	0.69	0.35	0.28	0.17	0.18	**0.009**
Stimulated salivary flow (ml/min)	0.42	0.33	0.49	0.4	0.56	0.51	0.39	0.43	0.41	0.296
Salivary pH	7.08	0.7	6.96	0.62	0.52	7.17	0.64	6.7	0.68	**0.03**
Salivary amylase	12455.9	5514.9	12717.5	5019.7	0.97	11452.3	5570.9	13211.9	4305.9	0.26

*Mann-Whitney test; SD=standard deviation

Table 3- Binomial negative regression model of number of surfaces with root caries, diabetes and salivary parameters.

		Unadjusted				Adjusted model 1				Adjusted model 2			
		Exp(B)	95CI		p	Exp(B)	95CI		p	Exp(B)	95CI		p
Diabetes	No	0											
	Yes	2.79	0.68	4.89	**0.009**	2.71	0.56	4.85	**0.013**	2.25	0.42	4.46	**0.046**
Unstimulated salivary flow		-2.55	-5.60	0.50	0.101								
Salivary pH		-0.87	-1.72	-0.03	**0.043**	-0.70	-1.67	0.27	0.15				

95CI = 95% Confidence interval of Wald

Model 1: Adjusted for controlled glycemic control and unstimulated salivary flow.

Model 2: Adjusted for age and unstimulated salivary flow.

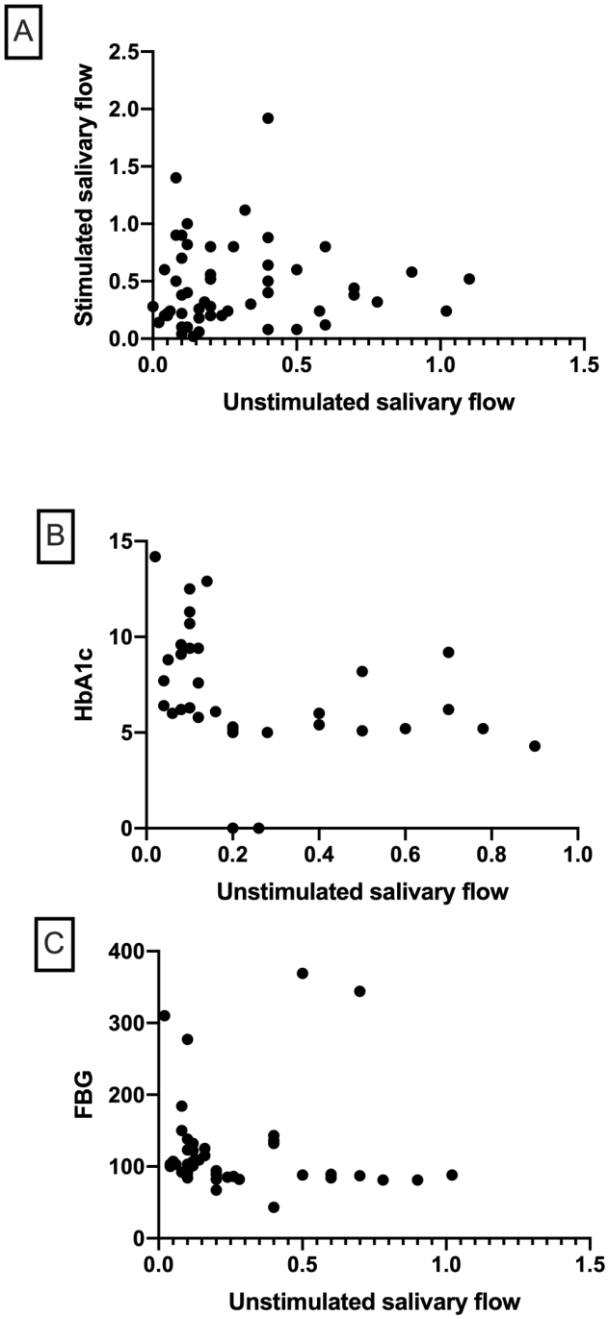


Figure 3. Correlations of the unstimulated salivary flow. A= Spearman correlation with stimulated salivary flow non-significant (r= 0.14; CI95%=-0.14 to 0.40; p>0.05); B= Spearman correlation with the glycated hemoglobin (HbA1c) (N=34; r=-0.57; CI95%= -0.77 to -0.28; p=0.0004); C= Spearman correlation with the fasting blood glucose (FBG) (N=34; r=-0.37; CI95%= -0.61 to -0.06; p=0.01).

3.5 DISCUSSION

We aimed to compare the salivary parameters of adults, exposed or not to DM, with and without active coronal caries and root caries. We could observe that chronic hyperglycemia caused by T2D affected their oral health, particularly the number of root caries, the salivary flow and pH. The unstimulated salivary flow, as well as the salivary pH were significantly affected by the glycemic control of T2D. Regarding caries, our findings show that individuals with T2D are 2.25 times more likely to have an increased number of root caries lesions when compared to those without DM. However, regarding the occurrence of active coronal caries (D-S), there was no difference between the control group and the DM group, partially accepting the null hypothesis that the caries activity would be influenced by the presence of DM.

Hyperglycemia induces altered inflammatory responses, which contributes to the systemic degradation of connective tissues, including periodontal ones, and decreases in oral pH (1). Prolonged hyperglycemia, in addition to causing systemic changes, can alter the function of salivary glands and can cause changes in the composition and volume of secreted saliva, due to polyuria or changes in the basement membrane of the salivary glands (12–14). In our results, although salivary flow did not show a significant difference between groups (Table 1), in the analysis of subgroups, individuals with uncontrolled glycemic control presented lower unstimulated salivary flow rate than the ones with controlled ones, in agreement with previous studies (10,15). Metabolic regulation seemed to play a significant role in saliva production but, interestingly, it was not observed for stimulated salivary flow, suggesting that the salivary glands, when stimulated, can be as efficient as the ones from controlled individuals. It might suggest a reasonability for using mechanical stimulation of the salivary glands as a treatment for xerostomia/hyposalivation in diabetics. Those findings were, at least in part, similar to what was found in the study by Carramolino et al (16), which showed a decrease in both basal and postprandial salivary flow levels in the DM group, although only significant differences were found in the case of basal resting salivary flow rate. Importantly, salivary flow is influenced by physiological factors and personal habits, such as taste, smell, and chewing (17,18). Chewing the latex device during stimulated collection may have

produced afferent signaling inducing response to increased flow in the affected population, compensating for the difference found in the assessment of flow at rest. This explains the importance of the collection method in the assessment of salivary flow, in order to prevent the use of stimuli in saliva collection from camouflaging the actual performance in salivary production of these patients. Standardizing assessment conditions, such as time and duration of collection, are also important to avoid possible assessment biases. In the present study, salivary flow measurement was performed in the morning (8 to 10 hours) to minimize the effect of circadian rhythms, and individuals were instructed to refrain from eating, drinking, and physical activity for at least two hours before the sialometry procedures.

Most studies on diabetes and caries suggest that the reduced salivary flow would increase the risk of caries due to the reduced protective effect of saliva directly on tooth surfaces (19–21). However, in the negative binomial regression model it was not possible to solely associate the increment of root caries lesions with the low salivary flow (Table 3). These results suggest that, even with the adjustment of the variables of unstimulated salivary flow and age, diabetes still explains part of the increase in caries lesions and this is not completely explained by the traditional classification of glycemic control (HbA1c≥6.5 and/or FBG>100). Furthermore, there were no statistically significant differences between the buffering capacity and the viscoelasticity of saliva in the T2D and control groups (Table 1), and these results on higher root caries extent cannot be isolated attributed to the quality of saliva either, as described in other studies (9,22). Changes in salivary pH in individuals with DM are also commonly reported and may represent a greater risk for the development of carious lesions (22,23). In hyperglycemia, a deficiency in insulin, which is a fundamental hormone for the regulation of blood sugar levels, results in an increase in other hormones such as glucagon and cortisol, that induce areas of the body that usually degrade sugars through the action of insulin, begin to degrade fats. It is this “fat-burning” to produce energy, due to the unavailability of sugars, which ends up producing ketone bodies that accumulate in the blood, and make the blood more acidic, with a drop in pH (24). An acidogenic environment with low salivary pH favors the growth of periodontal pathogens and aciduric bacteria, creating an inhospitable environment for protective oral bacteria, which can lead to the aggravation of

periodontal diseases and development of carious lesions, which further reduces the salivary pH, leading to a repetitive cycle (25). Here, the salivary pH was lower in individuals with HbA1c≥6.5 and/or FBG>100. Although we did not find its direct influence in caries, it should be better explored in further studies, with a more sensitive method for pH measurement.

Some hypotheses explain the increase in caries lesions in T2D. First, both caries and T2D share the same causal factor: a life of exposition to high carbohydrate intake. Studies using animal models strongly indicated that diabetic conditions enhance dental caries in mice and suggested a biological plausibility for this association (26,27). It was attributed to reduced salivary flow rates, the increased susceptibility to infection associated with DM, the impaired function of polymorphonuclear and leukocytes and, finally, diabetic animals typically have higher food intake than that of nondiabetic animals (27). In the analysis of the blood lipids levels, our results showed that the levels of triglycerides (lower in active caries) and HDL (higher in active caries) were significantly altered, and it might be related to the ingestion of saturated fat in individuals without active caries.

We observed that T2D can be risk factor for the increase in the number of root caries lesions, but we did not observe the same association between T2D with coronal caries. Both results are in agreement with a systematic review by Lima et al, which concluded that individuals with T2D are three times more likely to have root caries than non-DM and that there was no significant difference in the prevalence of caries between the DM group and systematically healthy controls (8). An explanation for these results would be that, with advancing age, root surfaces may be exposed due to periodontal diseases and mechanical instrumentation during scaling and root planing, facilitating the development of lesions in this region In addition, the susceptibility of the root surface to demineralization is greater than that of the coronal surface(28), which may have favored the greater development of lesions in the radicular region than in the coronal region (29) . Furthermore, it is already known that the diabetic collagen is altered (30,31), but the influence of hyperglycemia in the tooth collagen is unheard and could be another factor affecting root caries prevalence in DM individuals. In the negative binomial regression model, there was an important influence of age on these results, although the significance remained. The

cumulative effect of caries lesions over the years of life may explain the influence of age on this result.

Although the prevalence of coronal caries in T2D seems to contradict the literature (32,33), it is important to bear in mind that we considered only active D-S, excluding any past experience of caries and this is the main reason for the divergent results. Many studies use caries detection criteria that overestimate the disease activity in adults, such as the WHO-DMFT and DMFS, that take into account the extracted and filled tooth/surfaces. Although only extracted teeth due to caries should be recorded at DMFT/S in elderly patients and with diabetes, tooth loss due to periodontal disease is also common, and it is not possible to say which teeth were lost as a result of caries lesions. Considering the cross-sectional nature of our study, we used the Nyvad criterium, which assesses caries activity at the time of the examination and may be able to demonstrate a real correlation between dental caries and DM. Missing or restored teeth were not considered in our assessment in order to avoid confounding factors. The systematic review by Lima et al. concluded that although individuals with DM have a higher DMFT index than those without DM, both groups had similar caries prevalence (8), demonstrating the need for further longitudinal studies to better explore the complexity of risk factors involved in this relationship in adults, and agreeing with our results.

Although our study has allowed insights into the effects of T2D in the quantitative characteristics of saliva and the occurrence of caries, some limitations should be bear in mind when considering the results. First, an important difference in the age of DM and non-DM individuals should add some bias, although the adjusted regression models overcame the problem. Secondly, the measurement of pH and buffering capacity with an indicator strip (not very sensitive), but it seems that this is a simple cost-effective test for the clinical use as we demonstrated lower pH in individuals with uncontrolled glycemic levels. As this is an on-demand study, some patients did not show the complete blood tests examinations. Finally, the lack of a prior sample size calculation downrate the external validity of this study and further studies on coronal caries activity with higher sample size should be performed.

3.6 CONCLUSION

In conclusion, salivary parameters such as the reduction in unstimulated salivary flow do not explain itself the greater number of root caries lesions in patients with T2D. More factors are involved to explain the increased risk for root caries in those patients and this association should be evaluated longitudinally.

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4. CAPÍTULO 4 – DISCUSSÃO GERAL E CONCLUSÕES DA DISSERTAÇÃO

4.1 DISCUSSÃO GERAL

Com o aumento da expectativa de vida, tem se tornado comum pessoas adultas e idosas envelhecendo com uma maior quantidade de dentes presentes na boca. O avanço da idade pode vir acompanhado de doenças sistêmicas que podem causar impacto na saúde oral, como a Diabetes Mellitus. Diante deste novo cenário, torna-se necessário uma atenção maior à saúde bucal desta população, para garantir que estes envelheçam com qualidade. Estudos publicaram dados consistentes acerca da associação entre parâmetros salivares de indivíduos com DM comparados aos controles sem DM (1-4), sendo que nossa metanálise e estudo transversal demonstraram alterações significativas na saliva do DM, o que pode indicar a importância do diagnóstico e monitoramento desses parâmetros alterados nos indivíduos com DM, para a manutenção da saúde bucal.

Em relação à cárie, nossos achados mostram que indivíduos com DM2 têm 2,25 vezes mais chance de apresentar maior número de lesões de cárie radicular quando comparados àqueles sem DM2. Na análise dos subgrupos, os indivíduos com controle glicêmico não controlado apresentaram menor fluxo salivar não estimulado do que os controlados, em concordância com estudos anteriores (1,2). A regulação metabólica parece ter um papel significativo na produção de saliva, mas, curiosamente, não foi observada para o fluxo salivar estimulado, sugerindo que as glândulas salivares, quando estimuladas, podem ser tão eficientes quanto as de indivíduos controlados. Isso pode sugerir uma plausibilidade para o uso da estimulação mecânica das glândulas salivares como tratamento para xerostomia / hipossalivação em diabéticos.

Algumas hipóteses explicam o aumento das lesões de cárie em DM2. Primeiro, que essas doenças são consequências do consumo de carboidratos. Como citado no capítulo anterior, modelos animais indicaram uma combinação de alterações que podem explicar o aumento de lesões de cárie em Ratos com diabetes, e uma delas foi a maior ingestão alimentar (3,4). Esse aumento também foi atribuído à redução das taxas de fluxo salivar, ao aumento da

suscetibilidade à infecção associada ao DM, à função prejudicada de polimorfonucleares e leucócitos. Mais uma vez nossos resultados corroboram com a literatura, uma vez que detectamos níveis alterados de triglicerídeos (menor na cárie ativa) e HDL (maior na cárie ativa), podendo estar relacionado à ingestão de gordura saturada em indivíduos sem cárie. Nesse sentido, os indivíduos com DM podem ter mais energia advindas de carboidratos e isso deve ser melhor estudado no futuro.

Observamos que o DM2 pode ser fator de risco para o aumento do número de lesões de cárie radicular, mas não observamos a mesma associação do DM2 com a cárie coronária. Ambos os resultados estão de acordo com uma revisão sistemática de Lima et al, que concluiu que indivíduos com DM2 têm três vezes mais chance de ter cárie radicular do que não-DM e que não houve diferença significativa na prevalência de cárie entre o grupo DM e controles sistematicamente saudáveis, apesar do CPOD para cárie coronária ter sido maior em DM (5). Uma explicação para esses resultados seria que, com o avançar da idade, as superfícies radiculares podem ficar expostas devido a doenças periodontais e instrumentação mecânica durante a raspagem e alisamento radicular, facilitando o desenvolvimento de lesões nesta região (6). Além disso, a susceptibilidade da superfície radicular à desmineralização é maior do que a superfície coronal (7), o que pode ter favorecido o maior desenvolvimento de lesões na região radicular do que na região coronal. No nosso estudo, ainda propomos que a hipótese de que o colágeno alterado pela hiperglicemia (8,9) também poderia refletir no colágeno dentinário. Mais estudos são necessários para confirmar tal hipótese.

Na nossa metanálise, o fluxo salivar de pacientes com DM foi significantemente menor em pessoas com DM2 do que no grupo controle, tanto em repouso quanto estimulado. Também, apesar da maioria dos estudos demonstrarem que o pH no DM é inferior ao dos controles normoglicêmicos, não encontramos diferenças significativas. Já em nosso estudo transversal, o fluxo salivar não estimulado e pH foram significativamente afetados pelo controle glicêmico do DM2. Uma possível explicação para as diferenças encontradas entre os resultados da metanálise e do estudo transversal pode estar relacionada com o grupo amostral e com a idade mais avançada dos indivíduos com DM. Ainda assim, sugerimos que mais estudos são necessários para avaliar a

diferença do pH salivar. Uma padronização dos métodos de avaliação, sejam eles por fita ou por eletrodos, deverá ser proposta para que se alcance maior força de evidência.

Na avaliação da capacidade tampão, tanto a metanálise como o estudo transversal não encontraram diferenças significantes. Também não encontramos diferenças nas concentrações dos íons de cálcio e fósforo na metanálise, apesar desse resultado ser influenciado pelo pequeno número de estudos e seus tamanhos amostrais. Lembrando que esses componentes da saliva são essenciais para manter um equilíbrio na relação entre a mineralização e a desmineralização do esmalte (10) e níveis salivares de cálcio afetados em diabéticos, devido a secreção de insulina ser um processo cálcio-dependente, poderia afetar o equilíbrio mineral e ser mais um fator a explicar a maior prevalência de cárie em diabéticos, aqui demonstrada.

Como citado anteriormente, o aumento da ureia plasmática é um sinal básico de problemas renais e isso pode refletir na concentração de ureia na saliva. A nossa metanálise confirmou que a ureia salivar é significativamente maior em pacientes diabéticos. Sabendo que a nefropatia é comum em pacientes com DM, a ureia salivar parece ser um marcador futuro promissor no monitoramento de pacientes com nefropatia. Mais estudos são necessários correlacionando ureia sérica e ureia salivar, para verificar se a ureia salivar pode ser usada como biomarcador para indicar distúrbios sistêmicos, como a nefropatia.

4.2 CONCLUSÕES

Em conclusão, parâmetros salivares como a redução do fluxo salivar não estimulado não explicam por si só o maior número de lesões de cárie radicular em adultos com DM2. Mais fatores estão envolvidos para explicar o risco aumentado de cárie radicular nesses indivíduos e essa associação deve ser avaliada longitudinalmente, sendo que os mesmos podem incluir a dieta, o colágeno dentinário alterado pela hiperglicemia, a concentração alterada de íons cálcio e fósforo na saliva, o pH salivar alterado pela hiperglicemia (e não somente pelas bactérias orais), dentre outros fatores. Alterações significativas foram observadas em parâmetros salivares de indivíduos com DM, o que pode indicar a importância do diagnóstico e monitoramento desses parâmetros, especialmente o fluxo salivar e concentração de ureia, em todos os indivíduos com DM nos consultórios odontológicos.

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5. CAPÍTULO 5 – PRESS RELEASE

O indivíduo com Diabetes Mellitus tem a hiperglicemia sanguínea como característica principal que pode causar danos em diversos setores da saúde. Com o aumento da expectativa de vida, a população adulta que antes chegava na idade avançada edêntula, tem envelhecido com dentes em boca. Com isso, outras condições bucais passaram a ser motivo de estudo, para melhorar a qualidade de vida dessa população. Agravos Bucais, associados a Diabetes Mellitus vem sendo comumente relatados, como redução de saliva, perda de dentes, sensação de boca seca e cárie. Este estudo busca avaliar a associação entre as condições salivares de indivíduos com DM e indivíduos sem DM, na busca de melhor compreender as alterações que ocorrem no ambiente bucal. O primeiro estudo desta dissertação, buscou estudos publicados em diversas bases de dados, nacionais e internacionais que foram comparados por meio de análise estatística. Esse estudo mostrou que pessoas com diabetes mellitus apresentam menor secreção de saliva do que pessoas sem Diabetes Mellitus, e maior taxa de ureia na saliva. O fluxo salivar é importante para proteger a boca de infecções, além de ajudar na ingestão de alimentos. A taxa de ureia na saliva é importante, pois ureia elevada na saliva pode indicar problemas renais. O segundo estudo desta dissertação foi realizado no Hospital Universitário de Brasília com o objetivo de coletar dados sobre a correlação entre cárie e Diabetes Mellitus. Além disso, foi realizada coleta de saliva e análise em laboratório para determinar os padrões de parâmetros salivares. O estudo mostrou que existe pessoas com diabetes que tem menor produção de saliva, maior risco de ter cárie de raiz, e saliva mais ácida, e que o controle do diabetes pode estar diretamente ligado a essas alterações.

APPENDIX 1– Search strategies according to each database, performed on 13th March 2021 and updated in 25/05/21.

Appendix 1 – Search strategies according to different databases		
Pubmed	(((((("adult"[MeSH Terms] OR "adult"[All Fields]) OR "aged"[MeSH Terms]) OR "aged"[All Fields]) OR "elderly"[All Fields]) OR "middle aged"[MeSH Terms]) OR "middle aged"[All Fields]) AND ((((((((((((((((((((((("Diabetes Mellitus"[MeSH Terms] OR "Diabetes Mellitus"[All Fields]) OR "diabetes mellitus, type 1"[MeSH Terms]) OR "Insulin Dependent Diabetes Mellitus 1"[All Fields]) OR "Type 1 Diabetes Mellitus"[All Fields]) OR "Type 2 Diabetes Mellitus"[All Fields]) OR "Type 2 Diabetes"[All Fields]) OR "diabetes mellitus, type 2"[MeSH Terms]) OR "glycemic index"[MeSH Terms]) OR "Glycemic Control"[All Fields]) OR "Blood Glucose"[MeSH Terms]) OR "glucose control"[All Fields]) OR "Blood Glucose"[All Fields]) OR "Blood Glucose Monitoring"[All Fields]) OR "blood glucose control"[All Fields]) OR "blood sugar"[All Fields]) OR "advanced glycation end products"[All Fields]) OR "glycosylated hemoglobin"[All Fields]) OR "HbA1c"[All Fields]) OR ((((((((((((((("human s"[All Fields] OR "humane"[All Fields]) OR "humanely"[All Fields]) OR "humaneness"[All Fields]) OR "humanism"[MeSH Terms]) OR "humanism"[All Fields]) OR "humanities"[MeSH Terms]) OR "humanities"[All Fields]) OR "humanity"[All Fields]) OR "humanity s"[All Fields]) OR "humanization"[All Fields]) OR "humanize"[All Fields]) OR "humanizes"[All Fields]) OR "humanizing"[All Fields]) OR "humanness"[All Fields]) OR "humans"[MeSH Terms]) OR "humans"[All Fields]) OR "human"[All Fields]) AND ((("glycated hemoglobin a"[MeSH Terms] OR "glycated hemoglobin a"[All Fields]) OR ("hemoglobin"[All Fields] AND "a1c"[All Fields])) OR "hemoglobin a1c"[All Fields]) AND ((((("protein s"[All Fields] OR "proteinous"[All Fields]) OR "proteins"[MeSH Terms]) OR "proteins"[All Fields]) OR "protein"[All Fields])))) OR	1227 + 13

	"glycohemoglobin A"[All Fields]) OR "glycosylated hemoglobin A"[All Fields]) OR ((("glycosylated haemoglobin a"[All Fields] OR "glycated hemoglobin a"[MeSH Terms]) OR "glycated hemoglobin a"[All Fields]) OR "glycosylated hemoglobin A"[All Fields]) AND ("analysis"[MeSH Subheading] OR "analysis"[All Fields]))) OR "glycated hemoglobins"[All Fields]) OR "glycated hemoglobin"[All Fields]) OR "Blood Glucose"[All Fields]) OR "blood glucose metabolism"[All Fields]) OR "blood glucose analysis"[All Fields])) AND ((((((((((((((((("saliva/chemistry"[MeSH Terms] OR "salivary profile"[All Fields]) OR "salivary parameters"[All Fields]) OR "salivary pH"[All Fields]) OR "buffer effect"[All Fields]) OR "buffer capacity"[All Fields]) OR "salivary flow"[All Fields]) OR "salivary calcium"[All Fields]) OR "saliva calcium"[All Fields]) OR "salivary phosphate"[All Fields]) OR ((("saliva"[MeSH Terms] OR "saliva"[All Fields]) OR "salivas"[All Fields]) OR "saliva s"[All Fields]) AND (((((("phosphated"[All Fields] OR "phosphates"[MeSH Terms]) OR "phosphates"[All Fields]) OR "phosphate"[All Fields]) OR "phosphatic"[All Fields]) OR "phosphating"[All Fields]) OR "phosphation"[All Fields]) OR "phosphatized"[All Fields]))) OR "salivary urea"[All Fields]) OR "saliva/urea"[All Fields]) OR "phosphates/chemistry"[MeSH Terms]) OR "saliva calcium"[All Fields]) OR "saliva/urea"[All Fields]) OR "saliva"[MeSH Terms]) OR "saliva"[All Fields]) OR "salivas"[All Fields]) OR "buffers"[MeSH Terms]) OR "buffers"[All Fields])	
Cochrane	("adult" OR "aged" OR "elderly" OR "middle aged") in Title Abstract Keyword AND ("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose	184 Trials 1 review + 0

	control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar") in Title Abstract Keyword AND ("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers") in Title Abstract Keyword - (Word variations have been searched)	
LIVIVO	KW=(("adult" OR "aged" OR "elderly" OR "middle aged")) AND KW=(("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar")) AND KW=(("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers"))	153 (excluding Medline database) + 17
Scopus	(TITLE-ABS-KEY (("adult" OR "aged" OR "elderly" OR "middle aged")) AND TITLE-ABS-KEY (("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic	1456 + 40

	index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar")) AND TITLE-ABS-KEY (("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers")))	
Web of Science (13 de março de 2021)	TOPIC: ("adult" OR "aged" OR "elderly" OR "middle aged") AND TOPIC: ("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar") AND TOPIC: ("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers")	870 + 18
LILACS/BVS	(("adult" OR "aged" OR "elderly" OR "middle aged")) AND (("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin	19 +

	Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar")) AND (("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers"))	0
Google Scholar Web Search	("adult" OR "aged" OR "elderly" OR "middle aged") AND ("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar") AND ("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers")	18.400 resultados (only the first 209) +400
Proquest	noft(("adult" OR "aged" OR "elderly" OR "middle aged")) AND noft(("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control"	845 + 65

	OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar")) AND noft(("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers"))	
ScienceDirect	("adult" OR "aged") AND ("Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Glycemic Control") AND ("Saliva" OR "salivary pH" OR "salivary flow" OR "salivary calcium")	340 + 15
Open Grey	("adult" OR "aged" OR "elderly" OR "middle aged") AND ("Diabetes Mellitus" OR "Diabetes Mellitus Type 1" OR "Insulin Dependent Diabetes Mellitus 1" OR "Type 1 Diabetes Mellitus" OR "Type 2 Diabetes Mellitus" OR "Type 2 Diabetes" OR "Diabetes Mellitus Type 2" OR "glycemic index" OR "Glycemic Control" OR "Blood Glucose" OR "glucose control" OR "Blood Glucose" OR "Blood Glucose Monitoring" OR "blood glucose control" OR "blood sugar") AND ("Saliva/chemistry" OR "salivary profile" OR "salivary parameters" OR "salivary pH" OR "buffer effect" OR "buffer capacity" OR "salivary flow" OR "salivary calcium" OR "Saliva/calcium" OR "salivary phosphate" OR "saliva/phosphate" OR "salivary urea" OR "saliva/urea" OR "Phosphates/chemistry" OR "saliva/calcium" OR "saliva/urea" OR "saliva" OR saliva OR salivas OR "buffers" OR "buffers")	0 + 0

APPENDIX 2– Excluded articles and reasons for exclusion (n=34)

	Author, year	Reason for exclusion
After reading	*full-text*	
(1)	(Aitken-Saavedra et al. 2018)	7
(2)	(Banoczy et al. 1987)	1
(3)	(Carda et al. 2006)	1
(4)	(Dutra et al. 2014)	1
(5)	(Edblad et al. 2001)	1
(6)	(Ionescu et al. 1998)	6
(7)	(Kadir et al.2002)	1
(8)	(Koç et al. 2012)	1
(9)	(Yarat et al. 2012)	1
(10)	(Lima-Aragão et al. 2016)	1
(11)	(Malicka et al.2015)	1
(12)	(Martãnez et al. 2018)	1
(13)	(Miralles et al.2016)	1
(14)	(Mittal et al. 2016)	5
(15)	(Mohammed et al. 2019)	5

(17)	(Moore et al. 2001)	1
(18)	(My Tien et al. 2020)	1
(19).	(Nabi et al.2009)	1
(20)	(Närhi et al. 1996)	8
(21)	(Noboru et al. 2014)	1
(22)	(Panchbhai et al. 2010)	1
(23)	(Peres et al. 2016)	1
(24)	Pertruzzi et al.	1
(25)	(Reuterving et al. 1987)	1
(26).	(Sener A et al. 2009)	1
(27).	(Sharon et al. 1991)	1
(28)	(Shwetha et al. 2016)	1
(29)	(Sousa et al. 2011)	6
(30)	(Sreebny et al. 1992)	6
(31)	(Syrjala et al. 2003)	6
(32)	(Stancari et al. 1985)	6
(33)	(Tenovuo et al. 1985)	1
(34)	(Thorstensson et al. 2015)	1
(35)	(Thorstensson et al. 1995)	6
(36)	(Tiongco et al. 2019)	1

(37)	(Tremblay et al. 2012)	6
(38)	(Wakde et al. 2018)	6
(39)	(Willershausen et al. 1991)	6
(40)	(Yavuzyilmaz et al.1995)	1
(76)	(Younas et al 1996)	1
(77)	(Zloczower et al. 2007)	1
After methodological quality assessment:0		

1- Studies with children and adolescents, youth individuals, edentulous patients.
2 = Animals, *in situ* or *in vitro* studies;
3= Studies including systemic diseases or syndromes that can change the salivar flow (Individuals with Sjögren's Syndrome, patients who have undergone radiotherapy, individuals who have serious systemic complications, transplant patients, Hyper or Hypothyroidism, chemotherapy in the last 3 months, pregnant or lactating women and smokers).
4= Studies in languages not possible to be translated into an electronic translator;
5 = Articles with full text not available (Reviews, Book chapters, opinions, letters, conference abstracts, study protocols, case reports, case series) or duplicate data;
6 = Numeric data on either the sample or salivary parameters not specifically described (the author did not answer the protocol of data request, or denied sharing the data).
7 = Studies without control group (either no DM)
8 = Studies measuring salivary parameters other than biochemical, such as protein and immunological markers
10 = “No” answers in highly critical domains of the Joanna Briggs Institute instrument.

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APPENDIX 3 – Assessment of methodological quality of individual studies using the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies (Moola et al. 2020).

Author	1. Were the criteria for inclusion in the sample clearly defined?	2. Were the study subjects and the setting described in detail?	**3. Was the exposure measured in a valid and reliable way?**	**4. Were objective, standard criteria used for measurement of the**	5. Were confounding factors identified?	6. Were strategies to deal with confounding factors stated?	7. Were the outcomes measured in a valid and reliable way?	8. Was appropriate statistical analysis used?	**Overall appraisal:** LOW, MODERATE, OR HIGH QUALITY
Chávez, 2001	Y	Y	Y	Y	U	U	Y	Y	HIGH
Rahiotis, 2021	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Oyetola, 2019	Y	Y	U	U	U	N	Y	Y	LOW
Vasconcelos, 2010	Y	U	U	U	U	N	Y	Y	LOW
Bernardi, 2018	Y	Y	Y	Y	U	U	Y	Y	HIGH
Preejitha, 2019	U	U	U	Y	N	N	Y	Y	LOW
Masoumeh, 2013	Y	Y	Y	Y	Y	Y	Y	Y	HIGH

Prathibha, 2013	Y	U	U	U	Y	Y	Y	Y	LOW
Kogawa, 2016	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Malickaa, 2014	N	Y	Y	Y	N	U	Y	Y	LOW
AmbiK, 2018	Y	U	Y	Y	Y	Y	Y	Y	MODERATE
Meurman, 1998	Y	Y	Y	Y	Y	U	Y	Y	HIGH
Soares, 2004	U	U	Y	Y	U	N	Y	Y	LOW
Lopes, 2017	U	Y	Y	Y	Y	N	Y	Y	LOW
Kogawa, 2015	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Maheswari, 2016	U	N	Y	U	N	N	Y	Y	LOW
Rahiots	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Bernardi, 2007	Y	Y	Y	Y	Y	U	Y	Y	HIGH
Djuki, 2015	Y	Y	Y	Y	Y	U	Y	Y	HIGH
Carramolino	Y	Y	Y	Y	Y	Y	Y	Y	HIGH
Preethi, 2015	U	Y	Y	Y	Y	Y	Y	Y	HIGH

Pendyala, 2013	Y	Y	Y	Y	Y	Y	U	Y	HIGH
Mozaffari, 2019	Y	Y	Y	Y	Y	Y	U	Y	HIGH

Appendix 4- Assessment of methodological quality of individual studies using the JBI Critical Appraisal Checklist for Analytical Case Control Studies (Moola et al. 2020).

1. Were the criteria for inclusion in the sample clearly defined? **CRITICAL DOMAIN**
2. Were the study subjects and the setting described in detail? **CRITICAL DOMAIN**
3. Was the exposure measured in a valid and reliable way? **VERY CRITICAL DOMAIN**
4. Were objective, standard criteria used for measurement of the condition? **VERY CRITICAL DOMAIN**
5. Were confounding factors identified? **NON-CRITICAL DOMAIN**
6. Were strategies to deal with confounding factors stated? **NON-CRITICAL DOMAIN**
7. Were the outcomes measured in a valid and reliable way? **NON-CRITICAL DOMAIN**
8. Was appropriate statistical analysis used? **NON-CRITICAL DOMAIN**