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Dissertação de Mestrado

Saliva em adultos saudáveis e com condições associadas a hipossalivação

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Brasília, 18 de setembro de 2024

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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.

Orientadora: Nailê Damé-Teixeira

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RESUMO

Introdução: A saliva, um biofluido complexo com secreções mucino-serosas e contendo eletrólitos, proteínas e polinucleotídeos, é crucial para a saúde bucal e pode refletir alterações sistêmicas. Avaliar os constituintes salivares é essencial para entender, diagnosticar e manejar disfunções salivares. **Objetivo:** Caracterizar o fluido salivar em indivíduos saudáveis e naqueles com condições associadas à hipossalivação (HAC), em especial a Diabetes Mellitus. **Métodos:** Foram conduzidos dois estudos: uma revisão sistemática e um estudo transversal. A revisão sistemática foi relatada de acordo com o PRISMA. A busca envolveu oito bases de dados e literatura cinzenta e incluiu 69 estudos, num total de 3732 indivíduos saudáveis e 2875 apresentando alguma HAC. Fluxos salivares (ml/min) em repouso (USF) e estimulado (SSF) foram extraídos dos estudos e meta-analisados. Já o estudo transversal envolveu 170 indivíduos, dos quais 157 realizaram exames salivares, com 55 apresentando hiperglicemia (hemoglobina glicada - A1c ≥ 6,5%). Foram analisados fluxo salivar, pH, capacidade tampão, glicose, cálcio (Ca) e fosfato (Pi) salivares. **Resultados:** Na revisão sistemática, a média de USF foi 0,81 mL/min e o SSF foi 1,44 mL/min em indivíduos saudáveis. Indivíduos com HAC apresentaram diferença média de USF = 0,4 mL/min e SSF = 0,42 mL/min quando comparados com saudáveis. No estudo transversal, as médias de Ca, Pi e glicose na saliva foram, respectivamente, 8,71±6,12 mg/dL, 7,17±2,07 mg/dL e 31,99±15,38 mg/dL em hiperglicemia, e 11,09±6,52 mg/dL, 6,56±1,13 mg/dL e 26,88±18,80 mg/dL em controles normoglicêmicos. A glicose salivar foi negativamente correlacionada com o Ca (Rho=-0,42; p=0,000) e positivamente com o Pi (Rho=0,26; p=0,002). **Conclusão:** A caracterização do fluido salivar em indivíduos saudáveis versus aqueles indivíduos com HAC, como o Diabetes Mellitus, revela importantes diferenças na composição e no volume da saliva. Essas diferenças impactam a saúde bucal e o manejo dessas condições. Compreender a saliva em contextos normais, além dos patológicos, pode ajudar no desenvolvimento de estratégias eficazes de prevenção e tratamento de hipossalivação e boca seca, melhorando a qualidade de vida das pessoas adultas e idosas.

PALAVRAS-CHAVE: Saliva, fluxo salivar, glicose, cálcio, fosfato inorgânico, Diabetes mellitus, hipossalivação, revisão sistemática, estudo transversal.

ABSTRACT

Introduction: Saliva, a complex biofluid with mucinous-serous secretions containing electrolytes, proteins, and polynucleotides, is crucial for oral health and can reflect systemic changes. Assessing salivary constituents is essential for understanding, diagnosing, and managing salivary dysfunctions. **Objective:** To characterize salivary fluid in healthy individuals and those with hyposalivation-associated conditions (HAC), particularly Diabetes Mellitus. **Methods:** Two studies were conducted: a systematic review and a cross-sectional study. The systematic review was reported following PRISMA guidelines. The search involved eight databases and gray literature, including 69 studies with a total of 3,732 healthy individuals and 2,875 with HAC. Salivary flow rates (mL/min), unstimulated (USF) and stimulated (SSF) were extracted from the studies and meta-analyzed. The cross-sectional study involved 170 individuals, 157 of whom underwent salivary examinations, with 55 presenting hyperglycemia (glycated hemoglobin - A1c ≥ 6.5%). Salivary flow, pH, buffering capacity, glucose, calcium (Ca), and phosphate (Pi) were analyzed. **Results:** In the systematic review, the mean USF was 0.81 mL/min, and SSF was 1.44 mL/min in healthy individuals. Those with HAC showed a mean difference of USF = 0.4 mL/min and SSF = 0.42 mL/min compared to healthy individuals. In the cross-sectional study, the mean levels of Ca, Pi, and glucose in saliva were 8.71±6.12 mg/dL, 7.17±2.07 mg/dL, and 31.99±15.38 mg/dL in hyperglycemia, and 11.09±6.52 mg/dL, 6.56±1.13 mg/dL, and 26.88±18.80 mg/dL in normoglycemic controls. Salivary glucose was negatively correlated with Ca (Rho=-0.42; p=0.000) and positively with Pi (Rho=0.26; p=0.002). **Conclusion:** The characterization of salivary fluid in healthy individuals versus those with HAC, such as Diabetes Mellitus, reveals significant differences in saliva composition and volume. These differences impact oral health and the management of these conditions. Understanding saliva in both normal and pathological contexts may aid in developing effective prevention and treatment strategies for hyposalivation and dry mouth, improving the quality of life for adults and the elderly.

KEYWORDS: Saliva, salivary flow, glucose, calcium, inorganic phosphate, Diabetes mellitus, hyposalivation, systematic review, cross-sectional study.

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LISTA DE ABREVIATURAS E SIGLAS

sAA	Alfa-amilase salivar
A1c%	Hemoglobina glicada (em inglês)
BP	Bleeding on Probing
C	Grupo Controle
Ca	Cálcio
CT	Capacidade tampão
CRP	Proteína C-reativa
DM	Diabetes Mellitus
DM1	Diabetes Mellitus tipo 1 (em português)
DM2	Diabetes Mellitus tipo 2 (em português)
FBG	Glicose capilar
FCWBG	Glicose capilar (em inglês)
FSEG	Glicose Sérica
FSLG	Glicose Salivar
IgA	Imunoglobulina A
HbA1c	Hemoglobina glicada
HAC	Hipossalivação associada
MUC5B	Mucina - 5B
NA	Não aplicável
Pi	Fosfato inorgânico
pH	Potencial de hidrogênio
S-IgA	Imunoglogulina salivar
SFR	Fluxo salivar estimulado (em inglês)
USF	Fluxo salivar não estimulado (em inglês)
T1D	Diabetes Mellitus tipo 1 (em inglês)
T2D	Diabetes Mellitus tipo 2 (em inglês)
PC	Controle metabólico inadequado
WC	Bom controle metabólico

SUMÁRIO

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

1.1.INTRODUÇÃO

A saliva é um biofluido composto por uma secreção mucino-serosa e ligeiramente ácida, composta por eletrólitos, substâncias orgânicas, proteínas, peptídeos e polinucleotídeos [1], além de células descamadas e do microbioma oral. Sua secreção é feita, principalmente, pelos três pares de glândulas salivares maiores: parótidas, submandibulares e sublinguais, responsáveis por cerca de 90% da produção total de saliva diária [2]. As glândulas salivares menores encontram-se espalhadas por toda a mucosa bucal e também participam, porém em menores proporções, da produção salivar. O biofluido salivar é considerado um excelente material diagnóstico, pois as alterações nos seus componentes salivares podem ser reflexo de mudanças sofridas na composição sanguínea [3]. Além disso, a coleta desse fluido pode ser realizada de forma fácil e não invasiva [1, 4].

A redução do volume salivar pode resultar em hipossalivação. Não há exatamente um consenso na literatura, mas existe um ponto de corte para definir a hipossalivação quando o fluxo salivar em repouso é menor ou igual a 0,1 ml/min e/ou o estimulado é menor ou igual a 0,7 ml/min [5, 6]. A hipossalivação pode ou não estar associada à sensação de boca seca, denominada cientificamente por xerostomia, cuja queixa é frequente nos indivíduos com algum tipo de condição sistêmica capaz de acometer as glândulas salivares (Diabetes Mellitus, Síndrome de Sjögren, entre outras) [4, 7].

O envelhecimento pode interferir negativamente nas glândulas salivares, alterando a taxa de fluxo e a qualidade da saliva, incluindo a composição de íons e proteínas, bem como sua viscoelasticidade. Consequentemente, à medida que a pessoa envelhece, aumentam os sintomas de boca seca, as alterações no paladar e as chances de uma má higiene oral, que afetam significativamente a qualidade de vida desses indivíduos [8]. Além disso, também há favorecimento do desenvolvimento de alterações bucais, como a doença cárie, doença periodontal, candidíase, inflamação e alterações atróficas na mucosa oral, úlceras e infecções oportunistas [9]. Considerando que houve, ao longo dos anos, um aumento na expectativa de vida, esse fato também é relevante ao se pensar em alterações na qualidade e quantidade salivar. Com o aumento significativo da população idosa, espera-se que, nas próximas décadas, a população mundial com mais de 60 anos passe de 2 bilhões de pessoas até 2050 [10]. O interesse crescente em pesquisar a xerostomia tem sido impulsionado por esse aumento da população

idosa, muitas vezes acompanhado por aumento de doenças crônicas e polifarmácia, o que, por sua vez, tem contribuído para a secura bucal [11]. Visto que, os indivíduos com Diabetes Mellitus (DM) apresentam hipossalivação e/ou sensação subjetiva de boca seca, ou xerostomia. [12,13]. Fatores como tipo de fluxo salivar (repouso ou estimulado), os procedimentos e o momento de coleta, além da composição e origem das secreções (secreções menores ou maiores da glândula salivar), podem influenciar no relato desses pacientes sobre boca seca e sua relação com a hipossalivação [14, 15]. Portanto, a prevalência da xerostomia pode variar muito dependendo do método de avaliação e da população estudada [10, 16].

Além da presença de doenças crônicas, alguns medicamentos são conhecidamente indutores da xerostomia, como: drogas anticolinérgicas (atropinatropina, etc.), anfetaminas, antidepressivos (triclocarban, fenotiazinas e inibidores seletivos da recaptação de serotonina), drogas anti-histamínicas, diuréticos e agentes para controlar a pressão arterial [16].

Todos esses fatores que alteram a saliva são, portanto, modificadores da homeostase oral e de funções de defesa importantes para o organismo [10]. O equilíbrio da função salivar é crucial para a manutenção da saúde dos dentes, como podemos citar, por exemplo a presença de íons (Ca). Considerando que o Ca é um dos minerais mais abundantes no corpo humano [15] e tem sido intensamente estudado na saliva por seu papel na saúde bucal. No entanto, sabe-se que os níveis de íons de Ca são mais baixos em idosos saudáveis do que em adultos jovens saudáveis [10, 14].

Além do íon Ca, outros eletrólitos inorgânicos presentes na saliva, como os íons hidrogênio, fosfato inorgânico (Pi) e fluoreto, desempenham um papel importante no processo de remineralização, nos mecanismos de defesa, na atividade enzimática e na manutenção da estabilidade enzimática, dentre outras funções [14, 16, 17]. Os níveis desses íons na saliva atuam como ferramenta diagnóstica e terapêutica em diversas condições locais e sistêmicas, o que tem estabelecido a importância da avaliação dos constituintes salivares [17, 18].

Diante do exposto e dado que o equilíbrio oral depende da quantidade e qualidade da saliva, torna-se crucial estudar fatores salivares que alteram com idade e com doenças crônicas, bem como determinar o que se pode considerar normal. Esta dissertação apresentará dois artigos científicos. O primeiro capítulo apresenta o tema base a partir de uma revisão de literatura a respeito da relevância, composição e função da saliva na

saúde bucal e em condições associadas à hipossalivação (aqui apresentada com a sigla "HAC", do inglês "*Hyposalivation-associated conditions*").

O segundo capítulo apresenta uma revisão sistemática sobre o fluxo salivar em indivíduos saudáveis. A revisão foi realizada em conjunto com outros colegas de pós-graduação e está relacionada à disciplina de "Revisão Sistemática em Odontologia do PPGODT". Será apresentada em língua inglesa, pois objetiva-se submetê-la para publicação em periódico internacional.

O terceiro capítulo é um estudo transversal que realizou uma correlação entre os níveis de glicose salivar com os níveis de Ca e Pi. O artigo também será, futuramente, submetido a uma publicação internacional. O capítulo 4 apresenta a discussão geral do trabalho e os principais achados da tese, fornecendo ao leitor uma visão geral de como acredita-se que o trabalho possa colaborar no avanço dessa linha de pesquisa.

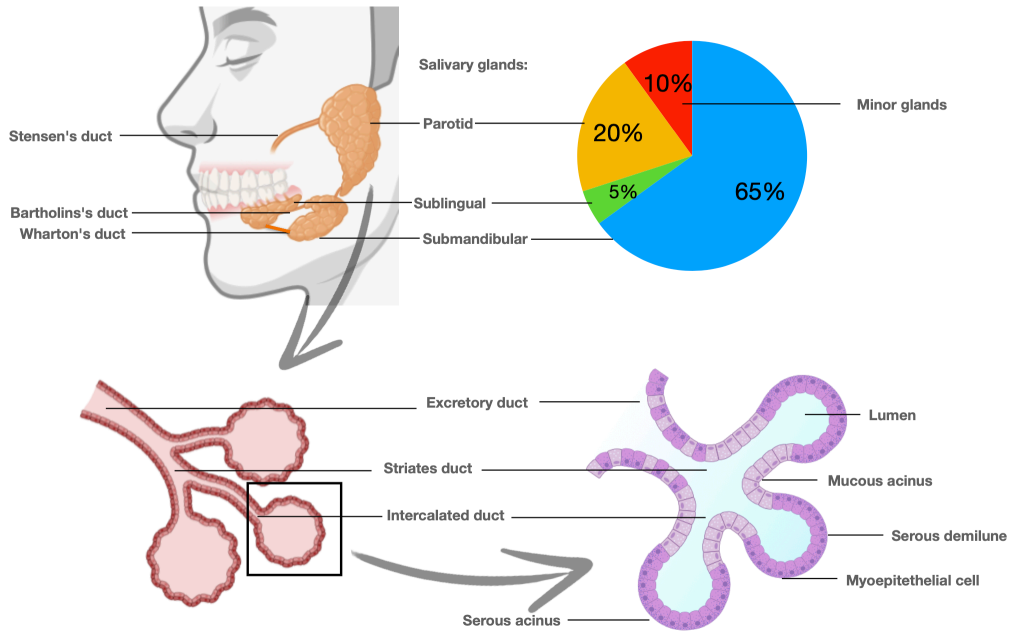
1.2.REVISÃO DE LITERATURA

1.2.1.Saliva

A saliva total é composta por água (99,5%), componentes inorgânicos (sódio, potássio, Ca, magnésio, cloreto, flúor, bicarbonato de nitrito, tiocianato e fosfatos) e orgânicos (proteínas, imunoglobulinas, enzimas, mucinas e produtos nitrogenados, como nitrito, nitrato, ureia e amônia) (0,5%), que são os principais responsáveis por manter os tecidos orais saudáveis [21-24]

A formação da saliva é realizada em duas etapas. A primeira etapa produz um líquido acinar primário, contendo íons e proteínas salivares. Na segunda etapa, as células dos ductos, sobretudo as estriadas, alteram a composição iônica e a secreção proteica do fluido acinar primário à medida que são secretadas na cavidade oral. O líquido acinar primário é hipertônico ou isotônico, enquanto a saliva é hipotônica [24]. As glândulas salivares maiores conectam-se com a cavidade oral através de seus respectivos ductos, bem descrito por Stensen, Wharton e Bartholin et al. (1989), e secretam diferentes tipos de fluido (parótida – principalmente serosa; submandibular – serosa mista e mucosa; sublingual – principalmente mucosa). As glândulas salivares são altamente vascularizadas e inervadas [25]. Essa intensa vascularização permite que biomoléculas e íons da corrente sanguínea penetrem nos ácinos das glândulas e sejam eventualmente incorporados à saliva. As secreções salivares são essenciais para funções vitais, como mastigação, lubrificação, articulação da fala e limpeza [25]. A inervação das glândulas salivares desempenha um papel crucial no controle dessas

secreções, assegurando a regulação eficiente dessas funções. A Figura 1 ilustra a localização anatômica das principais glândulas salivares maiores, além dos ductos salivares, células acinares e a produção do fluido acinar primário. A glândula submandibular é responsável por aproximadamente 65% do volume total de saliva excretada, seguida pela glândula parótida, que contribui com cerca de 20%. As glândulas salivares menores representam 10% do volume, enquanto a glândula sublingual produz apenas 5% do volume total de saliva.



Silva et al. 2024

Figura 1 - Representação esquemática das glândulas salivares e proporção que cada uma representa na saliva total excretada, adaptado de Rocca (2024), Taticonda (2020), Xu (2018), Varga (2015).

A principal via de estímulo para a produção de saliva é o sistema nervoso autônomo (SNA). O impulso nervoso do SNA, tanto parassimpático quanto simpático, pode influenciar tanto o volume quanto o tipo de saliva secretada, que pode ser serosa, mucosa ou mista. Quando estimulado, o sistema nervoso parassimpático aumenta as taxas de salivação e a secreção de enzimas [26]. Em contraste, o sistema nervoso simpático é responsável pela liberação de proteínas através da exocitose nas células acinares. Sua estimulação resulta na produção de saliva predominantemente mucosa, mais espessa, produzida principalmente pela glândula sublingual e, em menor grau, pela glândula submandibular [26, 27]. Tanto o aumento quanto a diminuição do fluxo salivar,

resultantes da ativação de um desses sistemas, podem afetar a concentração de substâncias no fluido salivar, como dos metabólitos e bioquímicos [28, 29]. Por isso, as medicações que influenciam diretamente nesses sistemas estão ligadas as as alterações salivares, como citado anteriormente.

A sialometria é um exame clínico utilizado para medir e avaliar a produção de saliva. Esse procedimento pode ser realizado em duas condições: com a saliva não estimulada e com a saliva estimulada. A saliva não estimulada mede a taxa de fluxo salivar quando o indivíduo está em estado de repouso, sem estímulos externos, resultando na avaliação da produção basal de saliva. Em contraste, a saliva estimulada é medida após a aplicação de estímulos mecânicos, como mastigação de goma, ou químicos, como ácido cítrico, permitindo a avaliação das glândulas salivares de aumentar a produção de saliva em resposta a estímulos [30]. Durante o exame, é possível avaliar diversas características da saliva: o fluxo salivar, medido em mililitros por minuto, indica o volume produzido; a viscosidade, que pode ser fluida, serosa ou viscosa; e a caraterização, que pode ser associada a diagnósticos. Essas medidas, principalmente o fluxo salivar em ml/min, essas medidas têm sido usada para diagnosticar e monitorar condições relacionadas à produção salivar, como xerostomia (boca seca) ou sialorreia (produção excessiva de saliva), e a eficácia dos tratamentos para essas condições [5, 30].

O diagnóstico de alterações bucais ou sistêmicas baseado na saliva tem atraído atenção significativa devido à sua facilidade de coleta, custo-efetividade, armazenamento acessível e não invasividade. Outros fluidos corporais, como sangue e urina, usados rotineiramente para diagnóstico de doenças têm problemas de coleta associados [23]. Aliado a esse conhecimento, sabe-se que a maioria dos componentes do sangue podem ser encontrados também na saliva, o avanço da biotecnologia tornou possível obter vários diagnósticos relacionados ao sistema fisiológico utilizando a saliva da cavidade bucal [24]. E, por isso, está sendo cada vez mais usada e validada como um biofluido para diagnosticar, monitorar e prevenir doenças. A complexidade desse fluido bucal talvez seja determinada por conta de seus diversos componentes e funções [31]. O termo "Salivomics" foi recentemente criado para determinar os principais constituintes da saliva: genômica, epigenômica, transcriptômica, proteômica e metabolômica [32]. Além disso, é possível incluir lipidômica e metagenômica microbiana - constituintes facilmente acessíveis. Em consonância, um estudo recente de Tomar et al. (2022) conclui que a saliva apresenta características semelhantes ao soro e outros fluidos corpóreos, pois contém biomoléculas como DNA, mRNA, microRNA, proteína, metabólitos e microrganismos [31].

1.2.2. Parâmetros salivares qualitativos

1.2.2.1.Valores de pH e tampão

Além do fluxo de salivar, a capacidade tampão da saliva e o conteúdo de saliva representam fatores muito significativos para a saúde bucal. O sistema tampão da saliva é responsável por manter o equilíbrio ácido-base adequado [33, 34]. As soluções de tamponamento mantêm um pH aproximadamente constante mesmo quando são adicionadas pequenas quantidades de ácido ou base, ou quando a saliva é diluída, sendo resistentes a alterações no pH oral.

Conforme descrito na revisão sistemática de Madariaga et al. (2023) [35], o pH médio intrabucal é de 7,4, e diminui ligeiramente durante o período de sono, atingindo cerca de um pH igual a 6. O pH crítico para a desmineralização do esmalte dentário está entre de 5,5–5,7 [36, 37], sendo que valores abaixo desse limite iniciarão a dissolução do esmalte. O pH crítico é definido como o pH limite em que há troca iônica igual entre um sólido imerso em sua solução saturada, não é um valor constante, e depende de outras características da saliva, como a concentração de Ca ou Pi [35].

Já a capacidade tampão (CT) também é um fator essencial para a manutenção da saúde bucal. Os tampões salivares têm a capacidade de neutralizar os níveis de pH ácido presentes no biofilme dentário, facilitando limpeza oral [38]. Consequentemente, esse processo ajuda a prevenir a desmineralização do esmalte dentário. Os valores são classificados da seguinte forma: CT normal (pH entre 5,0 e 7,0), CT limítrofe (pH entre 4,0 e 5,0) e CT baixo (pH inferior a 4,0) [37, 38]. Existem três sistemas tampão possíveis encontrados na saliva, nomeadamente: tampão proteico, tampão Pi e tampão ácido carbônico / bicarbonato.

Os tampões salivares são capazes de neutralizar o baixo pH do biofilme dentário e permitir a depuração oral, evitando assim a desmineralização do esmalte [38]. Por meio da sua capacidade tampão, a saliva também desempenha um papel importante na manutenção da integridade dos dentes (ao controlar a desmineralização, possibilitando a remineralização do esmalte e ao dificultar a erosão dentária) [37, 38]. A concentração de sistemas tampão (principalmente bicarbonato) aumenta com a taxa de secreção de saliva, e a capacidade tampão pode ser ineficaz no caso de baixo fluxo e quando a saliva é não estimulada [34].

O tampão bicarbonato é o sistema tampão mais importante da saliva. Porém, ele funciona essencialmente quando a saliva é estimulada, em que a concentração de bicarbonato é significativamente maior em comparação com a da saliva não estimulada

[39, 40]. Este íon surge da dissociação espontânea do ácido carbônico (H_2CO_3), a partir da reação entre CO_2 e H_2O (catalisada pela enzima anidrase carbônica). Há um equilíbrio dinâmico entre esses elementos, o que significa que a de gradiente se move em ambos os sentidos, dependendo do pH. Nesse sistema, o tamponamento ocorre quando os íons bicarbonato (HCO_3^-) são ligados aos íons hidrogênio (H^+), formando o ácido carbônico, que se dissolve em CO_2 e H_2O [39]. Assim tamponando o ácido produzido. O sistema tampão funciona na faixa de pH entre 5 e 7, a qual está determinada pelo pKa do H_2CO_3 (pKa = 6) Cury et al. (2017) [40], como descrito na fórmula abaixo:

$$CO_2 + H_2O <=> H_2CO_3 <=> H^+ + HCO_3^-$$

A influência do pH salivar no microbioma oral foi evidente em estudo recente do nosso grupo de pesquisa, refletida por alterações nas proporções da razão Firmicutes/Bacteroidota, que aumentou na saliva com pH alcalino. Vinte e três indivíduos diagnosticados com DM tipo 2 (DM2) e apresentando periodontite foram incluídos, assim como 25 indivíduos sistemicamente e periodontalmente saudáveis. Juntamente com a hiperglicemia sistêmica, o pH salivar teve maior número de táxons negativamente correlacionados entre todas as variáveis explicativas, superando inclusive a presença de lesões ativas de cárie [41].

1.2.2.2.Conteúdo orgânico

A saliva tem um papel crucial no processamento oral dos alimentos, assumindo, simultaneamente, uma função sensorial e uma experiência textural, além de ser extremamente importante na gustação, digestão e fala [23]. Os principais componentes da saliva que auxiliam no conforto oral são as enzimas, proteínas, glicose e ureia, pois estão intimamente relacionados à qualidade da lubrificação da cavidade oral.

A viscosidade salivar é uma característica inerente a esse biofluido e sofre influência direta de componentes salivares orgânicos, como mucina e albumina. As salivas mais viscosas têm impacto negativo na deglutição, mastigação, fonação e percepção do paladar [42]. Nesse sentido, o aumento da viscosidade na saliva de pacientes com HAC, como a DM mal controlada, pode ser atribuído à maior concentração dessas proteínas na saliva durante a hiperglicemia [43].

1.2.2.2.1.Glicose salivar

Alguns componentes salivares podem refletir o estado de saúde do corpo humano, e poderiam servir de biomarcadores para condições sistêmicas. O uso de glicose salivar como fluido diagnóstico para DM poderia ser um excelente exemplo. Alguns estudos mostram que, para pacientes com DM, o nível de glicose salivar está positivamente correlacionado com o nível de glicose no sangue, portanto, a glicose salivar poderia ser usada como marcador para detecção de DM [4, 44, 45].

Segundo Cui et al. (2022) [46], os níveis de glicose na glândula salivar parótida são maiores tanto nos pacientes com DM quanto nos controles, se comparados às concentrações encontradas nas glândulas sublinguais e submandibulares. Os níveis de glicose sérica e salivar da parótida dos pacientes com DM foram substancialmente mais altos em relação aos do grupo controle, e o nível de glicose salivar parótida estava fortemente associado ao nível de glicose sérica dos pacientes com DM [47]. Os níveis de glicose salivar no grupo de pacientes portadores do DM variaram de 0,67 a 4,31 mmol/L. Os níveis de glicose salivar no grupo controle variaram entre 0,51 e 3,02 mmol/L. Ademais, os achados de Cui et al. (2022) [46] confirmam que os níveis médios de glicose salivar são maiores na saliva não estimulada quando comparada à estimulada, tanto de pacientes saudáveis quanto com DM. Isso está relacionado à fase aquosa da saliva estimulada, que aumenta a secreção salivar, resultando em uma diluição da molécula de interesse [19, 47].

Em revisão sistemática com meta-análise, Naseri et al. (2018) [48] compararam o nível de glicose salivar de indivíduos portadores de 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 em relação ao grupo controle (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 entre os dois subgrupos [48].

As análises de glicose salivar, conduzidas previamente por nosso grupo de pesquisa, evidenciaram uma correlação positiva entre a glicose salivar e os níveis de A1c, glicemia em jejum e CPOD (número de dentes cariados, perdidos ou restaurados) em pessoas

com e sem DM2. Além disso, foi observada uma correlação entre a glicose salivar e o número de lesões de cárie coronária ativas em indivíduos com DM2. Vale ressaltar que, neste estudo, a glicose salivar foi medida a partir de saliva estimulada, utilizando um kit nacional de baixo custo (kit Labtest Glucose Liquiform®) [49]. Apesar dos estudos, essa correlação nem sempre é significativa e o uso de glicose salivar tem sido muito controverso [45].

1.2.2.2.2. Amilase salivar

A α-amilase salivar (sAA) é uma enzima predominante na saliva, responsável pela degradação do amido e do glicogênio em maltose [29], sendo sua principal fonte a glândula parótida [16]. Além disso, é um componente salivar com ação antimicrobiana que atua na imunidade inata da mucosa, impedindo a adesão e o crescimento bacteriano nas superfícies epiteliais [29].

A sAA desempenha um papel crucial na digestão do amido e na interação com a microbiota oral, contribuindo significativamente para a regulação da microbiota na cavidade oral [50]. A atividade desta enzima não apenas inicia a digestão do amido, mas também influencia a saúde bucal ao impactar a formação de biofilme. A digestão de amido pelos componentes salivares gera substratos que favorecem o desenvolvimento de placa e aumenta a adesão dos microrganismos às superfícies dentárias, potencializando a formação de biofilme [50, 51].

A sAA também pode se ligar à superfície dentária como componente da película salivar adquirida [52]. Níveis elevados de sAA na saliva indicam um ambiente microbiano favorável à formação de placa, embora não seja um biomarcador direto para microrganismos [53]. Os oligossacarídeos produzidos pela ação da amilase salivar podem atuar como moléculas aceptoras, viabilizando a formação de polissacarídeos extracelulares na matriz do biofilme dentário, viabilizando a adesão entre os microrganismos e a superfície dentária [52, 54].

Nos últimos anos, houve um interesse na integração da sAA para a pesquisa comportamental, de desenvolvimento e orientada para a saúde como um marcador substituto da ativação do sistema nervoso autônomo/simpático [55]. A sAA é uma das principais enzimas salivares presentes nos seres vivos e secretada pelas glândulas salivares sob estímulo simpático [56]. Dessa forma, a excitação simpática pode ser medida de forma rápida e não invasiva por meio da coleta de saliva, sendo, frequentemente, utilizada como um biomarcador adicional da excitação simpática. Além

disso, existe uma ligação positiva com os bloqueios adrenérgicos e com os β-bloqueadores; logo, sua atividade é medida durante situações de estresse, o que reflete a sua atuação simpática [56].

1.2.2.2.3. Mucinas salivares

As mucinas salivares são um conjunto de diferentes glicoproteínas de grande tamanho desempenham um papel fundamental na formação de revestimentos protetores que cobrem os tecidos dentários e a mucosa oral [29, 57]. Elas atuam como uma barreira funcional dinâmica capaz de reduzir os efeitos adversos do ambiente oral e são essenciais para os processos protetores que ocorrem no perímetro epitelial dos dentes e da mucosa [57].

Ademais, as mucinas são fundamentais para a viscoelasticidade do muco, impedindo o ressecamento das superfícies epiteliais. Ao controlar a permeabilidade da superfície da mucosa, limitam a penetração de agentes irritantes e toxinas nas células mucosas, protegendo as membranas das células mucosas das proteases produzidas pela microbiota dos biofilmes dentais. Sendo assim, acabam por regular a colonização da cavidade oral por microrganismos [58].

As mucinas são compostas por uma cadeia principal de polipeptídeos, com cadeias de oligossacarídeos ligados aos aminoácidos serina, treonina ou prolina. Os ácidos siálicos (ácido N-acetilneuramínico), fucose, N-acetilglicosamina e N-acetil-galactosamina são os mais abundantes, além de glicose, galactose e manose. As cadeias de oligossacarídeos podem se estender por toda a molécula ou estar localizadas em regiões específicas [59].

As cadeias de oligossacarídeos e os aminoácidos atuam em conjunto na determinação das propriedades bioquímicas das mucinas. A estrutura dorsal da mucina é composta por um número variável de repetições que contêm prolina, treonina e/ou serina (domínios PTS), além de regiões ricas em cisteína nas porções aminoterminal e carboxiterminal, intercaladas entre os domínios PTS [59, 60]. As mucinas salivares formam uma película estável (MUC1, MUC3, MUC4, MUC12) e uma película salivar móvel (solúvel secretado pelas mucinas: MUC2, MUC5A, MUC5B, MUC6 e MUC7) [8].

1.2.2.3.Conteúdo inorgânico

Dentre os eletrólitos salivares, o Ca, o Pi, o bicarbonato e o flúor são de grande importância para a saúde bucal. Tais íons possuem capacidade de tamponamento salivar e as concentrações iônicas de Ca e Pi são fundamentais na manutenção do equilíbrio entre a dissolução e a remineralização dos tecidos dentários [12, 20, 21]. Para avaliar o impacto da saliva na homeostase da cavidade oral, avaliar seus elementos bioquímicos pode ser relevante [4].

Por exemplo, muito se tem estudado sobre "Salivary Ionomics" em relação à doença cárie, considerando que o desequilíbrio de macroelementos, bem como macroelementos, poderia estar associado ao processo de desenvolvimento e/ou progressão da cárie [61]. Os macroelementos atuam na modulação de processos biológicos vitais, incluindo catálise enzimática, sinalização celular e produção de hormônios (a exemplo do sódio, potássio, Ca e magnésio). Outros oligoelementos são necessários em pequenas quantidades, incluindo iodo, selênio, ferro e zinco [61]. Tais elementos podem ser relevantes não somente para doença cárie, mas também para a homeostase microbiana. Por exemplo, diversas enzimas microbianas usam zinco como cofator. Consequentemente, o conteúdo iônico também pode influenciar outras doenças associadas ao microbioma oral, como periodontite e candidíase.

1.2.2.3.1.Ca e Fósforo salivar

Os eletrólitos salivares, como o Ca (Ca), o fósforo inorgânico (Pi) e o bicarbonato são fundamentais para a manutenção da saúde oral, uma vez que interagem com proteínas e células, fornecem substratos para a microbiota e atuam no tamponamento da saliva [19, 44, 47]. Além disso, como dito anteriormente, a concentração de íons Ca e Pi na saliva é indispensável para manter o equilíbrio entre o processo de desmineralização e de remineralização dos tecidos dentários [19, 44, 47]. Os tecidos dentários são compostos principalmente por minerais de hidroxiapatita [$Ca_{10}(PO_4)_6(OH)_2$]. Quando a saliva é subsaturada com Ca e Pi, ocorre um processo de desmineralização, a partir da dissolução do esmalte dentário. Por outro lado, a saliva é conhecida por estar supersaturada em relação a estes íons, depositando constantemente.

O Ca salivar pode estar associado ou não às proteínas salivares. Aproximadamente 20% do Ca salivar está fortemente ligado às proteínas que contêm estaterina ou prolina [62]. A quantidade de Ca ionizado e não ionizado está relacionada ao pH da saliva. À medida que o pH aumenta, o Ca livre estará na forma não ionizada e ligado a outros eletrólitos, como Pi e bicarbonato, citrato e outras macromoléculas [63, 64].

Combinando Ca ligado às proteínas, Ca ionizado e não ionizado, tem-se a concentração total de Ca na saliva, em torno de 2 mM [65]. A concentração de Ca tende a ser cerca de duas vezes maior na saliva secretada pelas glândulas submandibulares e sublinguais em comparação com a saliva secretada pela glândula parótida.

O Pi pode estar presente na saliva nas seguintes formas: H_3PO_6 (ácido fosfórico), $H_2PO_4^-$ (di-hidrogênio fosfato), HPO_4^{-2} (hidrogênio fosfato) e PO_4^{-3} (fosfato ou fosfato trivalente). Somadas, essas quatro formas representam o conteúdo total de Pi da saliva [66]. A concentração total de Pi na saliva diminui com o aumento do fluxo salivar. Na saliva não estimulada, a concentração de Pi total é de 5 mM, enquanto essa concentração reduz-se para 3 mM na presença do fluxo salivar estimulado [65]. Sendo assim, na saliva não estimulada a proporção de $H_2PO_4^-$ é maior, enquanto a proporção de HPO_4^{-2} na saliva estimulada depende do aumento no pH salivar em resposta ao estímulo [64, 66]. De forma geral, a secreção de fosfatos pelas glândulas submandibulares é cerca de três vezes menor quando comparada ao Pi secretado pelas glândulas parótidas. Uma parte muito pequena do Pi salivar pode estar organizada na forma de pirofosfato ($H_4P_2O_7$), o qual desempenha um importante papel de inibir a precipitação espontânea de Pi de Ca na superfície dentária, interferindo na formação de cálculo dentário [68].

Ca e fósforo estão presentes como componentes inorgânicos da saliva, que quantitativamente são os principais componentes minerais do sistema esquelético humano [69]. O fluido bucal tem potencial de avaliar as condições sistêmicas, detectar o início e a progressão da doença e monitorar resultados de tratamentos por meio de uma abordagem não invasiva [3].

O Ca está presente em diferentes formas no fluido oral, sendo grande porção encontrada ligada a componentes inorgânicos, como ortofosfato (H_3PO_4) ou carbonato ($CaCO_4$) ou macromoléculas, como proteínas. Porém, quase metade do Ca presente na saliva está em sua forma ionizada [65, 69, 70]. O Pi tem uma forte atração eletronegativa com íons de Ca. Aproximadamente 85% do Pi no corpo humano está nos ossos ou nos dentes, como hidroxiapatita (sal de Ca-Pi) [67]. Níveis salivares de Ca e Pi inorgânicos foram maiores em pessoas com periodontite quando comparados ao grupo de controles saudáveis e com doença cárie [31].

Devido às desordens metabólicas causadas pelo DM, os níveis de Ca na saliva dos portadores do DM podem ser alterados. Isso ocorre porque a secreção de insulina, que depende do Ca, reduz os níveis desse mineral no sangue, impactando, por consequência, os níveis salivares [16]. Além disso, maiores concentrações glicose

sanguínea estão relacionadas à maior excreção urinária de Ca e Pi, a qual é proporcional à glicemia [79].

Muitos estudos apontam que a composição da saliva em mulheres na menopausa com sensação de boca seca é diferente das mulheres na menopausa sem sensação de boca seca. Algumas dessas diferenças podem estar nos hormônios que estão relacionados à rotatividade óssea [80]. Foram investigados previamente os níveis de Ca, fósforo e fosfatase alcalina (marcadores bioquímicos comuns de renovação óssea) em soro e saliva estimulada e não estimulada de mulheres na menopausa. Os resultados mostraram que os níveis de Pi sérico, Ca salivar e fosfatase alcalina sérica foram significativamente mais elevados em mulheres na menopausa que sofrem de boca seca [69, 81].

O mesmo estudo constatou ainda que mulheres que se encontram na menopausa possuem o estrogênio significativamente menor, e Ca, paratormônio (PTH) e cortisol são mais altos em pacientes com sensação de boca seca em comparação com o grupo controle [80, 82, 83]. Não há consenso nos estudos que descrevem funções, concentrações e correlações dos níveis de glicose e dos íons Ca e Pi com os demais componentes salivares. Portanto, para melhor entendermos a relação entre a saliva e o equilíbrio dinâmico sistêmico e oral, é indispensável realizar uma avaliação detalhada das concentrações desses componentes salivares.

1.2.2.3.2.Saliva e condições associadas à hipossalivação (HAC)

Como dito anteriormente, em indivíduos saudáveis, a saliva desempenha um papel essencial na manutenção da saúde bucal e geral [84]. No entanto, a produção insuficiente de saliva pode comprometer significativamente suas funções. Condições que causam exocrinopatias como síndrome de Sjögren e efeitos colaterais da radioterapia sabidamente reduzem a produção de saliva [84-87]. Porém, outras condições diversas, locais ou sistêmicas, também podem levar a hipossalivação.

Desde a década de 80, já se afirmava que a diferença entre o fluxo salivar normal entre as pessoas saudáveis é grande, e, inclusive, há pessoas que não se queixam de boca seca, porém apresentam fluxo salivar reduzido. Dessa forma, um estudo da época já recomendava a inclusão da análise do fluxo salivar na rotina da clínica odontológica [88]. A análise do fluxo salivar deve ser feita de forma sistemática em todos os pacientes, pois os padrões e pontos de corte apresentados na literatura podem não ser úteis para

todos. Entretanto, é relevante comparar o paciente com ele mesmo e, dessa forma, perceber uma alteração precoce antes do aparecimento de sinais e sintomas relacionados à doença [89]. Uma média geral de como seria a saliva de pessoas normais possa ajudar a entender esse problema.

Um dos fatores mais comuns nos adultos e idosos que podem levar a alterações quantitativas e qualitativas da saliva é a DM [90]. Em revisão sistemática prévia, mostramos que indivíduos com DM tem taxas de fluxo salivar significativamente mais baixas, tanto na saliva não estimulada (diferença média [Md] = 0,13 mL/min) quanto na saliva estimulada (Md = 0,44 mL/min) [12]. Obviamente isso pode agravar o controle glicêmico, já que a saliva ajuda na digestão inicial dos carboidratos e no controle dos microrganismos orais acidogênicos [91, 92], prejudicando a primeira defesa antimicrobiana e o controle da esfoliação celular em decorrência da má lubrificação, entre outras repercussões [22, 93, 94].

Além da alteração no fluxo e na composição da saliva, citados acima, também é possível que ocorra diversas outras alterações nos componentes salivares que podem modificar os níveis de glicose e influenciar seu uso como biomarcador de hiperglicemia. Na Tabela 1 serão encontrados exemplos de referências que apresentaram significância estatística ($p<0,05$) para diferentes componentes salivares em pessoas com DM. Além disso, na mesma revisão sistemática citada acima, demonstramos que há redução significativa do pH e aumento da ureia salivar em DM [12]. Inúmeros fatores podem induzir distúrbios salivares em pessoas com DM. Algumas hipóteses são danos no parênquima glandular, alterações na microcirculação das glândulas salivares, desidratação e distúrbios no controle glicêmico. Outras alterações menos comumente estudadas em saliva de DM incluem variações nos níveis totais de proteínas, albumina, lisozimas, peroxidase, e eletrólitos como sódio, potássio, cloreto, Pi, magnésio e Ca. Além disso, podem ocorrer alterações na amilase, IgA e na capacidade tampão da saliva [4]. O impacto da DM na saliva será o tema de estudo no capítulo 3 da presente dissertação.

Tabela 1. Potenciais alterações causadas pelo o DM na saliva.

		Aumento ou diminuição significativa relatada na concentração	Referências
Componentes da saliva	Inorgânico	↑ Fosfato (Pi)	Moaffari et al. (2019), Ladgotra et al. (2016) e outros.
		↑ Cálcio (Ca^{2+})	Mrag et al. (2020), Ladgotra et al. (2016) e outros.
	Orgânico	↑ Amilase	Gonçalves et al. (2023), Pérez-Ros et al. (2021), Vandana et al (2021) e outros.
		↑ IgA	Shirzaiy et al. (2023), Olayanju et al. (2021), Omer et al. (2020), Hegde et al. (2020) e outros.
		↓ MUC1, MUC2, MUC5	Anwar et al. (2022), Chaudhurye et al. (2016) e outros.
		↑ Ureia	Marques et al. (2022), Seas et al. (2022) e outos.
		↑ Glicose	Cui et al. (2022), Pérez-Ros et al. (2021), Sharma et al. (2020), Kumar et al. (2020), Tiongco et al. (2019), Fares et al. (2019), Naseri et al (2019) e outros.

1.3.JUSTIFICATIVA

A saliva é fundamental para a preservação e manutenção da saúde bucal em adultos sistemicamente saudáveis, sendo importante revisar os parâmetros de normalidade atuais. Não está claro na literatura o volume médio de saliva necessário para manter a função oral normal. Além do mais, é de grande importância esclarecer a relação entre doenças sistêmicas, como DM, e as alterações nos componentes e volumes salivares. Diante do grande potencial de análise oferecida pela saliva e sua coleta ser simplificada e não invasiva, esta oferece uma alternativa ao sangue como fluido biológico para avaliar as condições sistêmicas. No entanto, a interpretação dos componentes inorgânicos, como Ca e Pi, é tão complexa quanto a análise proteômica, o que torna a avaliação dos metabólitos salivares um desafio. Este estudo tem potencial em auxiliar no entendimento de como a análise da saliva pode ser uma ferramenta na compreensão e diagnóstico de distúrbios orais e sistêmicos, dado que as alterações em sua composição frequentemente refletem o estado geral de saúde do indivíduo.

1. 4.OBJETIVOS

1. 4.1. Objetivo Geral

Avaliar quantitativamente e qualitativamente o fluido salivar em adultos sistemicamente saudáveis ou com doenças que potencialmente causam alteração do fluxo salivar, como o Diabetes mellitus.

1.4.2. Objetivos Específicos

1) Definir parâmetros de fluxos salivares em repouso e estimulado que possam ser compatíveis com a normalidade (Artigo 1);
2) Meta-analisar médias de fluxo salivar de pessoas adultas sem condições sistêmicas que acometem glândulas salivares (Artigo 1);
3) Comparar médias de fluxo salivar de pessoas adultas com condições sistêmicas que acometem glândulas salivares e controles saudáveis (Artigo 1);
4) Caracterizar a composição da saliva de indivíduos com e sem controle glicêmico, com foco específico em Ca e Pi (Artigo 2);
5) Investigar a possível relação entre esses parâmetros e os níveis glicêmicos, bem como outras condições clínicas em adultos (Artigo 2).

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2. CAPÍTULO 2 - REVISÃO SISTEMÁTICA

What is the normal salivary flow in healthy adults? A systematic review with metanalysis

2.1. ABSTRACT

Objectives: Hyposalivation negatively impacts individuals' health and quality of life. Common reference values to define hyposalivation are: <0.1 of unstimulated salivary flow (USF) and <0.5 of stimulated salivary flow (SSF). However, there is no evidence to confirm whether these parameters define normal conditions. The objective was to establish an average of USF and SSF in adults that could be used as a reference value for normality, as well to compare healthy individuals with the ones presenting any hyposalivation-associated condition (HAC).

Methods: This systematic review was reported according to PRISMA. Searches were performed across eight databases and grey literature. Inclusion criteria consisted of studies comparing the salivary flow rate in of a systemically healthy group with a HAC group. An effect size meta-analysis of average salivary flow in health was performed, as well as pairwise meta-analyses comparing the salivary flow between HAC and healthy individuals.

Results: Of the 6,407 titles retrieved, 69 were included in the analysis, reaching a total of 3,702 healthy individuals and 2,875 individuals with HAC. The participants were aged between 18 and 60, most female (60%). USF flow rates had an average of 0.81 (95% CI = 0.66-0.99; p = 0.0005) in healthy individuals, while SSF flow rates had an average of 1.44 (95% CI = 1.26-1.61; p = 0.005). The mean difference between individuals with HAC and the healthy ones was 0.4 (95%CI 0.04 to 0.76, p = 0.03, I^2 98%) for SSF and 0.42 (95%CI 0.14 to 0.71, p = 0.004, I^2 100%) for the USF.

Conclusions: The average salivary flow in healthy individuals is significantly higher than the current cut-off value used to define hyposalivation. Additionally, the difference between individuals with HAC and healthy ones is nearly half a microliter per minute. It is now important to validate saliva collection methods, and to consider seasonal temperature and humidity variations, as these factors can affect diagnostic accuracy and result reliability.

Keywords: Adults, Health, Salivary excretion, Salivary flow, Salivary elimination; Systematic review

2.2. INTRODUCTION

Saliva is essential for oral health and homeostasis, playing a vital role in host defense functions [1]. The most recommended clinical method for diagnosing salivary gland dysfunction is the quantification of total salivary flow rates, known as sialometry. This method involves assessing various characteristics of saliva, including flow rate (volume in milliliters per minute), color, turbidity, and viscoelasticity (whether fluid, serous, or viscous), evaluated both at unstimulated (USF) and stimulated (SSF) states through mechanical or chemical means [2, 3].

Despite its importance, accurate assessment of salivary dysfunction can be challenging due to considerable inter-individual variability in flow rates and the broad range of acceptable values [4]. For over 30 years [5-7], the cut-off values for normal salivary flow have been established as ≥ 0.1 ml/min for USF and ≥ 0.7 ml/min for SSF [8, 9]. However, results can vary depending on the type of saliva collected, the specific glands involved, the stimuli used for SSF, and other parameters [7]. Additionally, the choice of diagnostic device must consider saliva volume and the ability to assess clinical conditions. Various factors, such as age, diet, temperature, and pH, also influence salivary composition and flow rates [4, 10]. This complexity underscores the need for precise measurement techniques and a comprehensive understanding of factors affecting salivary function to ensure accurate diagnosis and management of salivary gland disorders.

Efforts have been made to establish a correlation between the perception of oral dryness and salivary production, with varying degrees of success. However, studies indicate a weak or negligible correlation between patients' subjective reports of oral comfort or discomfort and objective measurements of salivary flow rates [5, 6, 11]. Symptoms of dry mouth are often reported when salivation is reduced by approximately 50%, yet these symptoms can also occur within the normal range of salivary flow rates [6].

Chronic non-communicable diseases (NCDs) such as cardiovascular diseases, cancer, diabetes, and chronic obstructive pulmonary disease (COPD) are increasingly prevalent worldwide, affecting individuals across all age groups [12]. These diseases share common risk factors with oral conditions, including poor diet, smoking, and excessive alcohol consumption [12-14]. Individuals with NCDs are more likely to experience oral health problems, and poor oral health can further complicate the management of these chronic conditions, negatively impacting overall well-being [12-14]. Poor oral health, characterized by dental caries, periodontal disease, and tooth loss, can alter eating

habits, leading to frailty and dependency, and adversely affect quality of life [15]. A significant contributing factor to poor oral health is reduced salivary volume [12].

The importance of saliva is often not fully appreciated until it is absent, by which time damage to the salivary glands can be substantial and potentially irreversible. Early diagnosis of hyposalivation could enable the use of conservative stimulation methods, such as chewing gum or physical stimulation, to mitigate adverse effects. The objective of this study was to establish normative values for salivary flow rates in adults, which could aid in the early detection and effective management of hyposalivation, thereby enhancing oral health and overall quality of life.

2.3. METHODS

This systematic review was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) checklist [16]. The protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) platform under identification number CRD 449389.

Search strategies

The following databases were searched: PubMed/MEDLINE, Embase, LILACS, Web of Science, Scopus, Science Direct, Cochrane, and Livivo, as well as the gray literature, ProQuest for dissertations and theses, OpenGrey, Google Scholar, and reference lists. The main terms added in the search strategy to assess the “adult” or “adults”, “aged”, “elderly” or “elderlies”, "salivary elimination”, "salivary flow”, "salivary excretion” or “Sialometry”. Both MeSH terms and free terms were included. The complete search strategy can be found in Appendix 1 in the supplementary material. The search date for all the databases was July 8, 2023.

Inclusion criteria

Studies eligible for this review were observational studies (cross-sectional, retrospective, and/or prospective cohort and case-control studies) or interventional studies (clinical trials, randomized or not), with no restriction of publication period or language limitations. Observational and clinical studies, without time or language limitation, in which a group of healthy adult individuals performed sialometry. Studies with adult populations (over 18 and under 60 years old) were included when they assessed the salivary profile, salivary flow in ml/min and with salivary stimulation by mechanical means.

Exclusion criteria

Studies were excluded if (1) studies without a systemically healthy group; (2) studies with children, adolescents or elderly; (3) animal or *in vitro* studies, articles with an unavailable full text,reviews, book chapters, opinions, letters, conference abstracts, study protocols, case reports, or case series; (4) studies that reported stimulation for SSF measurement using acids or othernon-mechanical stimuli; (5) studies that did not report the mean or median salivary flow combined with a standard deviation or a p value; (6) studies that did not specify the age range of the individuals included.

Selection process

Two independent reviewers (J.R.S. and R.C.R.) searched for studies and selected the titles and abstracts of each study based on the eligibility criteria. Conflicts were resolved by consensus with a third reviewer (F.N.). Subsequently, the same reviewers independently assessed the full texts, confirming their eligibility. Discrepancies were resolved with the involvement of the third reviewer. Rayyan QCRI (Qatar Computing Research Institute, Qatar, freely available at https://www.rayyan.ai) was used during both phases of the study selection.

Data collection process

Qualitative analysis was carried out on the data obtained from each study, including the first author, the year of publication, the country, the sample size of systemically healthy group (without any oral or systemic comorbidities that can cause hyposalivation) and a group having some hyposalivation-associated conditions or systemic disorders, the proportion of men among the healthy subjects and the control subjects, the average age of the systemically healthy group and the control group -subjects, the methods for collecting saliva and the salivary flow rates (ml/min).

Quantitative synthesis

Quantitative synthesis was performed by grouping studies that reported mean and standard deviation results in ml/min. For studies that did not report a standard deviation, but did report a mean and a p-value, the standard deviation was calculated using the online calculator [18].

An effect size meta-analysis of healthy participants data was carried out using the mean salivary flow (unstimulated and stimulated) and the standard error of each included study, using the maximum likelihood method and the random effects model on JASP software [19].

Pairwise meta-analyses were conducted for comparing the salivary flow (stimulated and unstimulated) between hyposalivation-associated (HAC) and healthy individuals using the mean difference (Md) as the measure of effect size, Mantel-Haensel and Dersimonian and Laird method random effects model, using RevMan 5.41 software [20]. Confidence was stablished at 95%, and the heterogeneity was assessed through I^2 statistics and Cochran's Q test.

2.4. RESULTS

Selection of studies and assessment of methodological quality

A total of 15669 studies were identified by searching the databases. After exclusion of duplicates, 6407 studies were identified for screening, of which 5441 were excluded based on the PECO question (population, exposure, comparator, outcome) to define the inclusion criteria. Of the remaining 923 studies, 854 were excluded based on the exclusion criteria. A total of 69 studies met the inclusion criteria, with 49 studies evaluating unstimulated saliva [21-69] and 28 studies evaluating stimulated saliva [22, 31, 33, 39, 43, 48, 53, 54, 56-58, 63, 65, 69-81] used for the meta-analysis. Figure 1 shows PRISMA flowchart depicting the identified, included, and excluded studies with reasons

Table 1 shows the descriptive characteristics of the 69 studies included in the qualitative synthesis. The studies were published between 1976 and 2023 and were widely distributed geographically according to Figure 2.

A wide range of HAC groups were included. From these, 11 studies included groups with diabetes mellitus (prediabetes, type 1 and type 2 diabetes) [38, 42,50, 52, 54, 68, 78, 80, 87, 93, 94], 4 included groups with dental caries [35, 39, 46, 57], and 3 with periodontitis [36, 40, 73]. Other HAC included mucosal lesions [21, 53], bulimia nervosa [33, 74], smoking [37, 49], rheumatoid arthritis [27, 83], asthma [84], history of xerostomia [63], chemically-dependent subjects [62], chronic alcoholics [95], hematopoietic stem cell transplantation [92], malaria [96], menopause [86], tuberculosis [66], multiple sclerosis [61], tooth wear [97], temporomandibular disorders [60], presence of volatile sulphur compounds [32], coeliac disease [75], use of oral contraceptives [72], fasting condition [24], and chronic mental illness [23] (Table 1).

In total, there were 3,702 healthy subjects and 2,875 subjects with a local or systemic condition that caused reduced salivary flow (HAC). The 49 studies measured SSF in healthy subjects (ml/min) and 28 studies measured SSF (ml/min). In addition, 35 studies

compared a healthy group with a HAC group (Table 1).

Quantitative synthesis

The meta-analyses comparing averages of salivary parameters for healthy individuals is shown in Figure 3A (USF) and Figure 3B (SSF). Mean USF was 0.81 ± 0.6 (95%CI 0.65 to 0.98, I^2 99.9%), while mean SSF was 1.44 ±0.45 (95%CI 1.26 to 1.62, I^2 99%). The mean difference for the salivary flow between individuals with HAC and healthy individuals was 0.4 (95%CI 0.04 to 0.76, P= 0.03, I^2 98%) for the USF (Figure 4A) and 0.42 (95%CI 0.14 to 0.71, P= 0.004, I^2 100%) for the SSF (Figure 4B).

2.5. DISCUSSION

The aim of this study was to establish an average healthy salivary flow rate in adults that could be used as a reference value for normality, as well to compare healthy individuals with the ones presenting any hyposalivation-associated condition (HAC). We identified this the average is 0.82 ml/min (USF) and 1.44 ml/min (SSF) in healthy conditions and less than 0.4 ml/min (USF/SSF) in conditions that cause hyposalivation.

The first average value used for USF was proposed by Becks et al. in 1943 [98]. At that time, the average USF in healthy individuals was 0.32 ml/min (SD ± 0.23), and this nowadays it is still used as reference value. However, the methods to evaluate saliva volume has changed over the years. Also, in that study, a very heterogeneous sample was included. For example, individuals between 5 and 95 years of age were included in the calculations. It is important to note that the salivary glands appeared to be fully developed after 15 years [94]. Also, any potential factors altering the salivary flow was evaluated [99]. The average values for SSF in adults date back to 1983 [100]. After mechanical stimuli with the chewing of 'paraffin wax', the average value of salivary flow was 1.6 ml/min (SD ±2.1). However, there are other protocols of salivary stimulation, for example using citric acid (1 to 3%) [4], food (usually acidic drinks), chewing gums, and even rubber rubber dams. All these methods can generate different degrees of stimuli, so the parameters should be different to all of them. Nevertheless, the lack of standardisation in saliva collection methods is the main challenge in diagnosing hyposalivation.

The considerable heterogeneity observed between included studies should be taken into account. For instance, a range of 0.16 ml/min to 2.39 ml/min for unstimulated salivary flow (USF) was reported. Some studies indicated mean values exceeding 2 ml/min [23, 39, 46, 114], while others reported values below 0.2 ml/min [24, 33, 54] for USF in healthy

individuals. Similarly, the mean stimulated salivary flow (SSF) in healthy individuals ranged from 0.43 ml/min to 2.10 ml/min, with some studies showing mean values under 0.5 ml/min [63, 78] and others exceeding 2 ml/min [39, 43, 77]. This significant variability in reported values can be attributed to changes in salivary collection and stimulation methods over time, as well as differences in sample characteristics, such as age, gender, and sample size. This lack of standardization in collection and stimulation protocols likely contributes to the absence of a uniform reference value, leading to discrepancies in the reported average salivary flow and impacting the accuracy of diagnoses and interpretation of conditions associated with hyposalivation.

The method of saliva collection was a crucial factor in the selection of studies for this analysis. Only studies that used mechanical stimulation (chewing) and reported salivary flow in ml/min were included. Of the 69 studies evaluated, 68 collected saliva in the morning between 8 am and 12 pm, while only one study collected saliva in the afternoon, from 2 pm to 6 pm [77]. Studies that chemically stimulated saliva production or reported flow rates in g/min were excluded. Thus, the standardized method for stimulation was the collection of stimulated whole saliva after chewing paraffin (1 g) for 1 to 5 minutes, with the volume recorded and expressed in milliliters per minute. However, this parameter was not taken into account and the values were not converted into ml/min. Some studies have assumed that the density of saliva is approximately the same as that of water, suggesting that 1 g of saliva is approximately equivalent to 1 ml [3, 5, 21, 22].

These findings underscore the need to reconsider the diagnostic cut-off points for hyposalivation. The variability in values observed suggests that current thresholds may not accurately reflect the true range of normal salivary flow, potentially leading to false negatives in diagnosing early-stage salivary volume issues and affecting the interpretation of xerostomia versus hyposalivation. Additionally, misguided or inadequate interventions may result from these misdiagnoses, as the observed variations in salivary flow among healthy individuals may not align with the standards commonly used in clinical practice and dental research.

It is currently acknowledged that a comprehensive assessment of salivary parameters must integrate both traditional and contemporary considerations [1, 2]. This necessitates the consideration of contemporary lifestyles and their impact on health, cultural diversity, local habits, as well as environmental, geographical, and climatic factors specific to a given region. As detailed in recent literature [3, 4], the objective of this study was to evaluate a population of adults aged 18 to 60 years in order to more accurately

characterise the study cohort and mitigate confounding factors associated with systemic alterations and chronic medication use, which can adversely affect salivary production.

Normal salivation vs. HAC

Among the main conditions associated with hyposalivation, diabetes, including prediabetes, Type 1, and Type 2 diabetes, is a significant focus. Studies have shown that individuals with diabetes often have lower unstimulated and stimulated salivary flow rates compared to healthy controls [5-15]. Diabetes can exacerbate oral health issues, such as dental caries, due to factors like increased salivary glucose [16]. However, xerostomia in diabetic patients might be more related to peripheral neuropathy rather than a true decrease in saliva production [5].

Other conditions linked to hyposalivation include dental caries, periodontitis, and mucosal lesions. Studies on dental caries have highlighted the importance of salivary parameters like USF and SSF in oral health [17-20]. Periodontitis has been associated with increased salivary protein production but decreased flow rates [21-23]. Mucosal lesions, such as oral lichen planus (OLP), often result in low salivary secretion due to altered gland function or medication use [24, 25]. Conditions like bulimia nervosa and smoking also impact salivary flow, with bulimics showing lower flow rates and smokers experiencing adverse effects from tobacco use [26, 27] [28, 29]. Additionally, rheumatoid arthritis may reduce salivary flow, likely due to xerogenic medications rather than the disease itself [30][31]

In addition to the commonly studied conditions linked with hyposalivation, various other factors significantly impact salivary flow, each with unique implications. Asthma, for instance, is often managed with inhaled corticosteroids, which can lead to dry mouth and persistent salivary flow issues [32]. Similarly, substance abuse - encompassing both illicit drugs and prescription medications - along with chronic alcohol consumption, contributes to impaired salivary function due to their dehydrating and toxic effects on the salivary glands [33, 34]. Hematopoietic stem cell transplant recipients frequently experience salivary dysfunction as a result of their conditioning regimen [86]. Moreover, conditions such as malaria, menopause, tuberculosis, and multiple sclerosis also affect salivary function through their systemic impacts or treatments [35 - 38]. Temporomandibular disorders and the presence of volatile sulfur compounds in the mouth are further indicators of reduced salivary flow and associated oral health issues [39,40]. Celiac disease and the use of oral contraceptives can alter salivary flow due to disease effects or hormonal changes [41,42]. Additionally, fasting results in decreased salivary flow from

reduced fluid intake [43], while chronic mental illness influences salivary production through medication side effects and stress [44].

As previously outlined, hyposalivation can be attributed to several factors that directly influence salivary production and flow. Xerogenic medications, often used in the treatment of conditions such as rheumatoid arthritis and asthma, have been shown to reduce salivary flow. Systemic conditions and therapies, such as diabetes and chemotherapy, also have a detrimental effect on salivary gland function. Additionally, lifestyle habits including substance abuse, tobacco use, and excessive alcohol consumption contribute to dehydration and impaired salivary function. Specific diseases, such as oral lichen planus and celiac disease, as well as environmental and behavioral factors like fasting and mental stress, have also been shown to affect salivary secretion. Therefore, hyposalivation results from a complex interplay of behavioral, systemic, and environmental factors that impact salivary production and flow.

Strengths and limitations of this systematic review

The strengths of this study include, firstly, the identification of average salivary flow rates for healthy adults, establishing valuable reference values for unstimulated salivary flow (USF) and stimulated salivary flow (SSF) at 0.82 ml/min and 1.44 ml/min, respectively. These values serve as important benchmarks for assessing normal salivary function. Additionally, by comparing healthy individuals with those presenting conditions associated with hyposalivation (HAC), the study contributes to a clearer understanding of the impact of these conditions on salivary flow, providing a practical threshold of less than 0.4 ml/min for diagnosis. The study also addresses variability in salivary flow measurements across different studies and recognizes the need for standardized protocols to enhance accuracy and consistency. Furthermore, it offers practical insights to improve diagnostic precision and the effectiveness of interventions for hyposalivation and related conditions.

Among the limitations of this study are the variability in collection protocols and the lack of rigorous standardization among methods, which may have introduced variability in the data and affected the comparability of results, along with potential measurement errors. Environmental and behavioral factors, such as diet and substance use, may also have impacted the results, and the study may not have adequately addressed variations associated with specific conditions, such as autoimmune diseases or cancer treatments. There is a clear need for ongoing data updates to reflect changes in clinical practices and new discoveries. Lastly, the lack of uniform control over environmental conditions and participants' lifestyle may have introduced additional variability. These limitations

highlight the need for future studies with standardized methodologies and more homogeneous samples to enhance the accuracy of salivary flow data and its relationship with hyposalivation.

2.6. CONCLUSION

The average salivary flow in healthy individuals is significantly higher than the current cutoff value used to define hyposalivation. Additionally, the difference between individuals with HAC and healthy ones is nearly half a microliter per minute. It is now important to validate saliva collection methods, and to consider seasonal temperature and humidity variations, as these factors can affect diagnostic accuracy and result reliability.

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TABLES AND FIGURES CAPTIONS

Table 1. Characteristics of the included studies according to the hyposalivation-associated condition (HAC) groups and healthy groups (N = 69 studies).

Study	Number of healthy	Number of HAC group	Disease	Age HAC (MEAN ±SD range) year old	Age healthy (Mean ±SD range) year old
Abd Ali et al. 2020 Iraq	22	36	multiple sclerosis	37.2±4.3years	38.7±6.8 years
Aframian et al. 2006 Israel	50 (M-26 F-24)	NA	NA	NA	29 ± 8 years
Aggarwal et al. 2015 USA	80 (M- 40 F-40)	NA	NA	NA	20-50 years
Agha-Hossein et al. 2016 Iran	40 (M- 8 F- 32)	40 (M- 8 F- 32)	oral lichen planus	47.73 ± 11.06 years	43.25 ± 10.89 years
Arhakis et al. 2013 Greece	38	NA	NA	NA	25.21 ± 5.06 years
Atalay et al. 2019 Turkey	50	50	Tooth wear	45.82 ± 9.88 years	45.82 ± 9.88 years
Atif et al. 2023 Italy	109 (M- 47 F- 62)	NA	NA	NA	M - 29 (24–39) years F- 25.5 (20–36) years
Bardow et al. 2014 Denmark	85	170 : 1- Erosion (n = 85) 2- Caries (n = 85)	Erosion and Caries	1- 48 ± 15 years 2- 48 ± 14 years	47 ± 15 years
Belardinelli et al, 2014 Argentina	30 (G1. G2. G3)	NA	NA	NA	G1- 21-30 years G2- 31-40 years G3- 51-60 years
Brasil-Oliveira et al. 2020 Brazil	50 (M- 26 F- 24)	40 (M- 6 F- 34) severe 35 (M- 5 F- 30) mild-to-moderate	asthma	severe 51.8 ± 10.8 years. mild-to-moderate 42.5 ± 14.2 years	48.2 ± 12.4 years
Caroline et al. 2015 France	216	NA	NA	NA	49.6 ± 13.5 years
Chakrabarty et al. 2015 India	30	60	smoker	1- 32.6 ±5.27 years 2- 32.06 ±5.58 years	31.96 ±6.88 years
Chhugani et al. 2021 India	30 (M- 15 F-15)	30 (M- 19 F-11)	history of xerostomia	52.1±11.6 years	46.9±9.2 years
Cortelli et al. 2014 Brazil	30 (M- 12 F- 18)	30 (M- 12 F- 18)	type 2 diabetes	49.23 ± 9.41 years	49.23 ± 9.41 years
Dezayee et al. 2016 Iraq	25 (M- 7 F- 18)	T1D 25(M-7 F- 18) T2D 50(M-18 F-32)	patients with T1D and T2D	33.16 ± 3.77 and 50.86 ± 5.066	37.12 ± 5.622
Doddawad et al. 2022 India	30	30	Tuberculosis	48.90 ± 9.324 years	43.20 ± 13.935 years
Dukić et al. 2013 Croatia	70(M-55 F-15)	70 (M-58 F-12)	CHRONIC ALCOHOLICS	41.7 ± 9.26 years	39.1 ± 8.78 years
Dynesen et al. 2008 Denmark	23	24	bulimia nervosa	18-33 years	20-30 years
Emekli-Alturfan et al. 2008 Turkey	11	20	NA	Average 52 years	Average 45 years
Evaristo-Chiyong et al. 2021 Peru	153	NA	NA	NA	40.1±17.6 years
Galvão-Moreira et al. 2018 Brazil	70 (F-46 M-24)	NA	NA	NA	18-40 years
Hajisadeghi et al 2023 Iran	13	13	diabetic	51.00 ± 10.12 years	33.53 ± 12.71 years

Table 1. Characteristics of the included studies according to the hyposalivation-associated condition (HAC) groups and healthy groups (N = 69 studies) (continued).

Hajisadeghi et al 2023 Iran	13	13	diabetic	51.00 ± 10.12 years	33.53 ± 12.71 years
Hoek et al. 2002 United Kingdom	40	NA	NA	NA	32 ± 11 years
Inoue et al. 2006 Japan	50 (M-24 F-26)	NA	NA	NA	M- 26.0 ± 2.7 years F- 24.4 ± 3.2 years
Javed et al. 2015 USA	50	45	Prediabetes	42.6±3.2years	44.5±3.6
Jawed et al. 2010 Pakistan	395	398	type 2 diabetic	30-50 years	30-50 years
Jawed et al. 2013 Pakistan	300	400	type 2 diabetic	40.94 ± 9.68 years	41.55 ± 11.25 years
Jentsch et al. 2004 Germany	28 (M- 12 F-16)	NA	NA	NA	23.5 ± 2.1 years
Justino et al. 2017 Brazil	14 (M- 7 F- 7)	NA	NA	NA	21 ± 2 years
Kanzow et al. 2023 Germany	39	NA	NA	NA	31.8 ± 13.7 years
Kim et al. 2011 Korea	10 (M)	30 (M)	smokers	26.5 ± 4.1 years	27.4 ± 3.4 years
Kogawa et al. 2016 Brazil	38	72 (1- 36 / 2- 36)	type 2 diabetic	1- 57.50 years 2 - 56.50 years	51 years
Koss et al. 2009 Argentina	29 (M-14 F-15)	89	Periodontal disease	1- 42.6 ± 0.6 years 2-40.8 ± 10.5 years 3- 47.2 ± 11.2 years	45.9 ± 10.6 years
Laaksonen et al. 2011 Switzerland	144 (M-69 F-75)	228 (M-134 F-94)	hematopoietic stem cell transplantation	± 43 years	± 46 years
Lähteenmäki et al. 2000 Finland	11 (M)	NA	NA	NA	21±29 years
Laine et al. 1991 Finland	21 (M- 10 F- 11)	11 (F)	oral contraceptives	24.7 ± 2.9 years	M- 31.2 ± 3.7 years F- 29.9 ± 3.4 years
Larrucea et al. 2013 Chile	37	NA	NA	NA	19 - 23 years
Lasisi et al. 2012 Nigere	20	20(M- 10 F-10)	DIABETIC	1- 62.6 ± 9.1years 2- 54.2 ± 10.7 years	3- 55.3± 7.0 years 4- 45.1± 8.5 years
Lasisi et al. 2015 Nigeria	50 (M-15 F- 35)	40 (M- 13 F- 27)	Malaria	29 ± 7.73 years	29.76 ± 7.51 years
Lenander-Lumikari et al. 2000 Finland	30	30	coeliac disease	42.7 ± 14.7 years	39.12 ±12.7 years
Lester et al. 2021 UK	30 (M- 9 F- 21)	NA	NA	NA	18-40 years
Ligtenberg et al. 2016 Netherlands	20	NA	NA	NA	18–32 years (± 25 years)
Lyra et al. 2020 Brazil	27	27	Chemically dependent subjects	18-50 years	18-50 years
Mahesh et al. 2014 India	20	40 (F)	Menopausal	1- 51.40±1.96 years 2- 49.75±1.92 years	39.60±2.95 years
Miralles et al. 2006 Spain	90	90	type 1 diabetics	18 - 50 years	18 and 50 years
Mladenovic et al. 2018 Bosnia and Herzegovina	30	45	Temporomandibular Disorders	26.3 ± 4.5 years	27.3 ± 4.9 years
Mobarak et al. 2011 Egypt	40	40	caries experience and oral hygiene	20-30 years	20-30 years

Table 1. Characteristics of the included studies according to the hyposalivation-associated condition (HAC) groups and healthy groups (N = 69 studies). (continued).

Nagler et al. 2003 Israel	18 (M -4 F-14)	34 (M-8 F- 26)	rheumatoid arthritis	51.0 ± 2.0 years	46.6 ± 4.6 year
Nishihara et al. 2014 Japan	64 (M- 39 F- 25)	NA	NA	NA	24.8 ± 2.3 years
Oliveby et al. 1989 Canada	5	NA	NA	NA	26-38 years
Ono et al. 2007 Japan	50 (M-24 F-26)	NA	NA	NA	M- 26.0 ± 2.7 years F- 24.4 ± 3.2 years
Öztürk et al. 2008 Turkey	21 (M-10 F-11)	16 (M-8 F-8)	Carie	21.2 ± 1.8 years	(20.3 ± 1 years)
Öztürk et al. 2012 Turkey	20	23	type 2 diabetic	45.3 ± 13.5 years	48.4 ± 9.3 years
Pereira et al. 2016 Brazil	12 (M- 3 F-9)	NA	NA	NA	20 - 25 years old
Prester et al 2017 Croatia	25 caries-free controls	30 dental caries-active	caries-active	31 (22–40) years	35 (20–40) years
Rahim et al. 1991 United Kingdom	19 (M-10 F-9)	22(M- 12 F- 10)	Fasting	23 and 61 years	23 and 61 years
Ramesh et al. 2021 India	130 (M- 65 F-65)	NA	NA	NA	G1- (20– 29 years; n=42) G2- (30–39 years; n= 48) G3- (40–49 years; n= 40)
Rayment et al. 2001 USA	6 (M)	NA	NA	NA	30-36 years
Rytomaa et al. 1998 Finland	105	35	Bulimia	25.3 +- 6.8 years	25.7 ± 7.0 years
Sánchez et al. 2011 Argentina	15 (M-14 F-1)	45	periodontitis patients	1- 30–46 years; 2- 35–57 years . 3- 32–61 years	27–41 years
Sánchez et al. 2011 Argentina	15 (M-14 F-1)	45	periodontitis patients	1- 30–46 years; 2- 35–57 years . 3- 32–61 years	27–41 years
Sewón et al. 1990 Finland	46 (M-18 F-28)	NA	NA	NA	± 24.2 years
Sewón et al. 1995 Finland	15	25	Periodontitis	38 - 55 years	37 - 56 years
Shetty et al. 2013 India	80	NA	NA	NA	20-30 years
Shirzaiy et al. 2015 Iran	25 (M- 8 F- 17)	25 (M- 8 F- 17)	type 2 diabetic	30 - 45 years	30 - 45 years
Sopapornamorn et al. 2007 Japan	18 (M-5 F-13)	67 (M-20 F-47)	Volatile sulfur compounds	47.8 ± 1.7	41.6 ± 1.5
Stiefe et al. 1990 USA	29 (M- 19 F-10)	37 (M-24 F-13)	chronic mental illness	33.4 ± 8.6 years	30 ± 8.6 years
Torres et al. 2016 Brazil	38 (M-12 F- 29)	104 (M-12 F-92)	RA	± 53 years	± 47 years

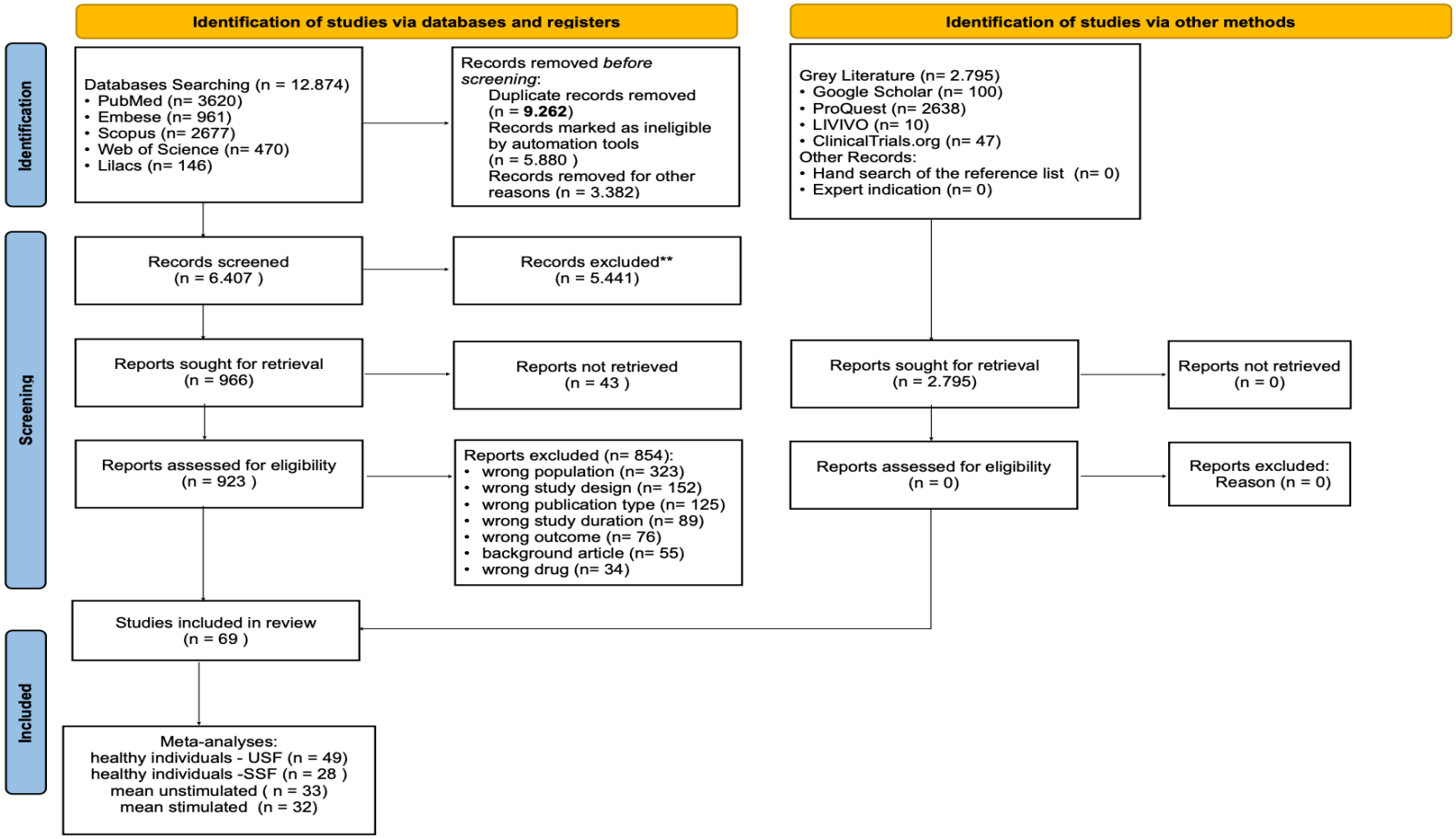


Figure 1. PRISMA flowchart describing identified, included, and excluded studies with reasons. *Databases searched included PubMed/MEDLINE, Embase, LILACS, Web of Science, Scopus, Science Direct, Cochrane, and Livivo. **Registers included ProQuest for dissertations and theses, OpenGrey, Google Scholar, and reference lists.)

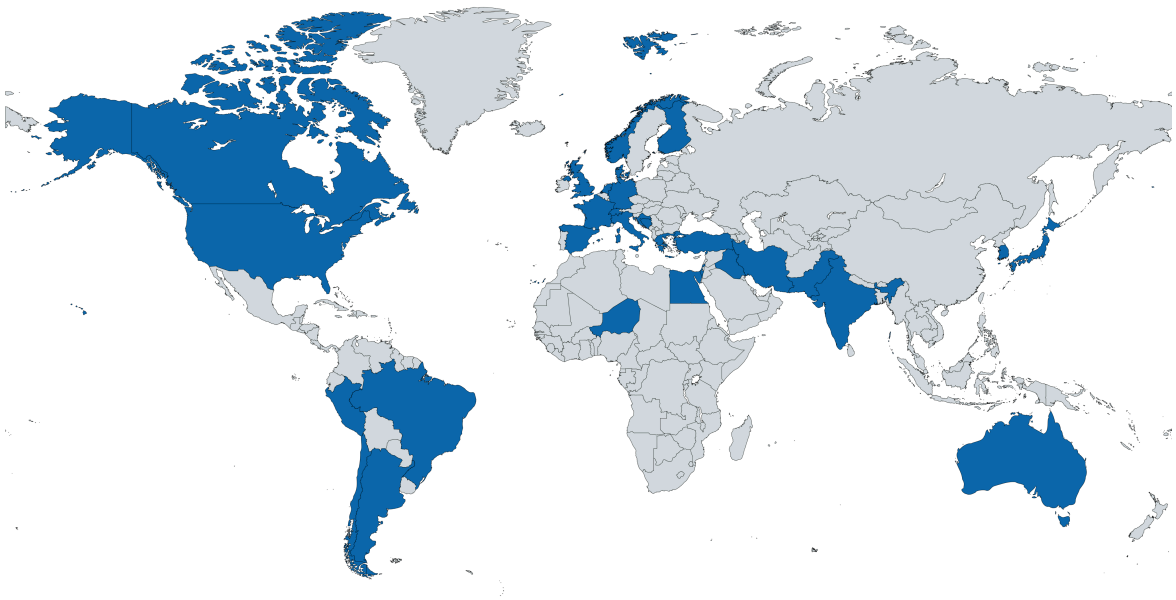
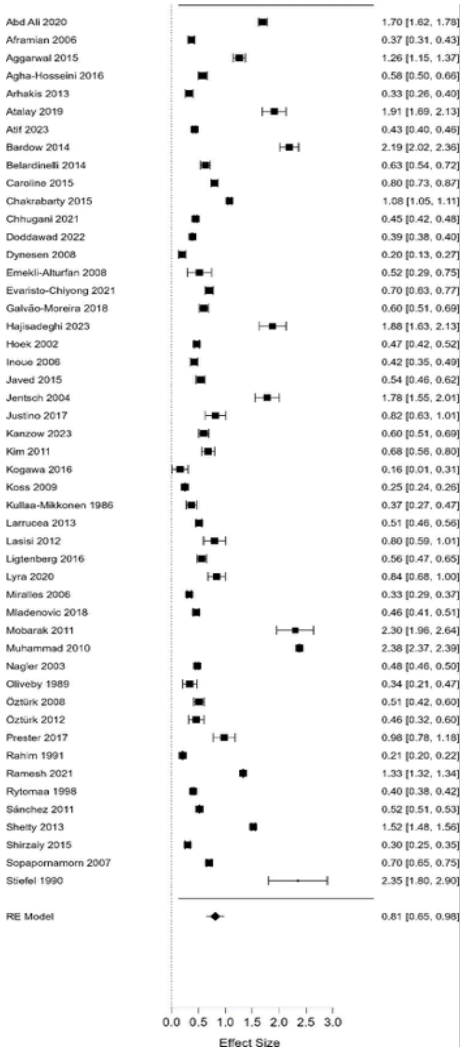


Figure 2. Geographical distribution of include studies: Brazil, Finland, India, Turkey, Japan, USA, Argentina, Iran, United Kingdom, Croatia, Denmark, Germany, Iraq, Israel, Netherlands, Pakistan, Bosnia and Herzegovina, Canada, Chile, Egypt, France, Greece, Italy, Korea, Norway, Peru, Spain, Switzerland.

A



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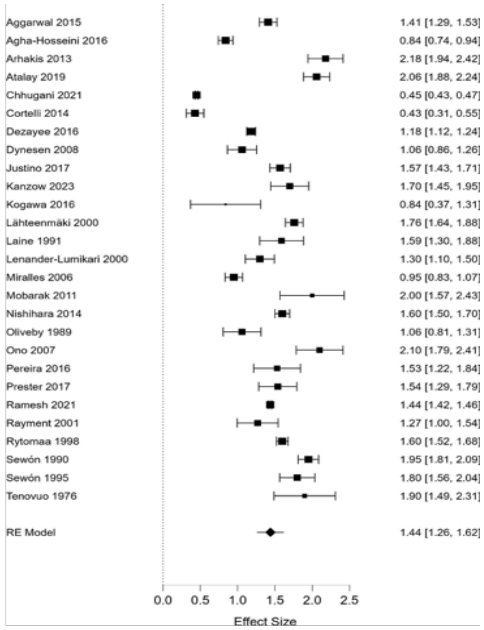
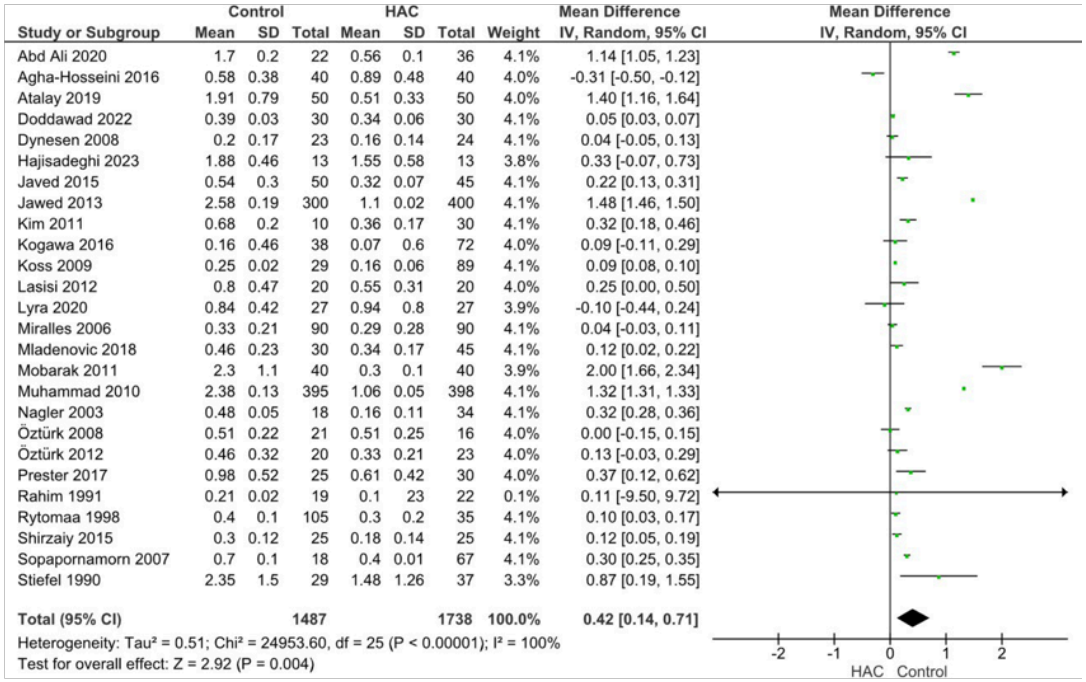


Figure 3: Effect size meta-analysis with the mean salivary flow. A) Unstimulated salivary flow (USF); B) Stimulated salivary flow (SSF).

A



B

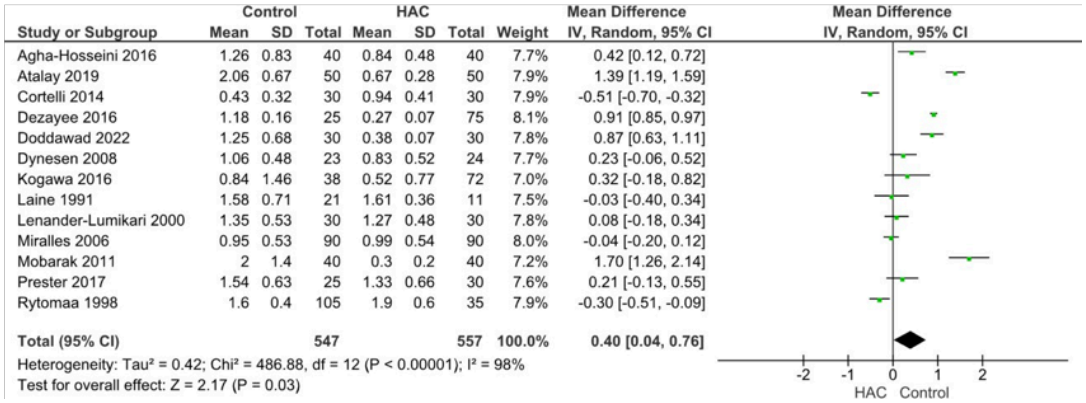


Figure 4. Pairwise meta-analysis Hyposalivation-Associated Conditions (HAC) versus healthy. A) Unstimulated salivary flow (USF); B) Stimulated salivary flow (SSF).

3. CAPÍTULO 3 - ESTUDO TRANSVERSAL

Correlations of salivary glucose, calcium and phosphorus levels: a cross-sectional study

3.1. ABSTRACT

Objectives: This study aimed to characterize the inorganic salivary composition of hyperglycemic and normoglycemic individuals, focusing specifically on calcium (Ca) and inorganic phosphorus (Pi), and to investigate their potential correlations with glycemic levels and other clinical parameters.

Methods: A cross-sectional study was conducted including dentate adults (>35years-old). Blood and saliva (rest and stimulated) samples were obtained on the same day to assess glycemic levels. Salivary Ca and Pi concentrations were quantified using spectrophotometry from the stimulated saliva. Statistical analyses were performed comparing averages between groups, as well as using Pearson / Spearman's correlation and Linear regression models ($p<0.05$ significance level).

Results: Out of 170 individuals that comprised our sample (53.49±11.14 years-old), 157 performed saliva tests, from which 55 had glycated hemoglobin (A1c) ≥ 6.5%. Averages of salivary Ca, Pi, and glucose in saliva were 8.71±6.12 mg/dL, 7.17 ± 2.07 mg/dL, 31.99±15.38 mg/dL for individuals in hyperglycemia, and 11.09 ± 6.52 mg/dL, 6.56±1.13 mg/dL, 26.88±18.80 mg/dL for normoglycemic controls, respectively. Salivary glucose was negatively correlated with Ca (Rho=-0.42; p=0.000), and positively correlated with Pi (Rho= 0.26, p=0.002). Salivary flow, normoglycemic status or diagnosis of DM did not significantly impact this correlation.

Conclusions: Alterations in the inorganic composition of hyperglycemic saliva, particularly in Ca levels, may impair its protective mechanisms, leading to an increased risk of ionic imbalance and, consequently, an increased incidence of caries development. Saliva has the potential to be a practical and accessible tool for monitoring systemic conditions, and in individuals with DM could be useful as an oral health risk assessment.

Keywords: Diabetes Mellitus; Adults; Saliva; Salivary glucose; Calcium; Phosphorus; cross-sectional

3.2. INTRODUCTION

According to the World Health Organization (WHO), the number of individuals diagnosed with diabetes mellitus (DM) has quadrupled over the past 40 years [1]. DM is the single major non-communicable disease whose risk of early mortality is increasing, rather than decreasing. The WHO further predicts that approximately 500 million individuals worldwide will be affected by DM by 2030 [1, 2]. Type 2 diabetes mellitus (T2D) is resulted of a chronic high blood glucose levels due to insulin deficiency or insulin resistance leading to hyperglycemia [3, 4]. Long-term uncontrolled hyperglycemia can cause complications such as neuropathy and microvascular abnormalities (endothelial dysfunction and deterioration of the microcirculation), while a proper control of DM can delay or prevent serious complications [2, 5].

Complications of T2D are closely linked to changes in the oral cavity. Elevated glycated hemoglobin (A1c) levels can impair salivary gland function, leading to reduced salivary flow due to hormonal, microvascular, and neuronal changes [4, 6-8]. This decrease in salivary flow due to acute hyperglycemia can lead to various oral changes, including increased salivary glucose concentration, higher prevalence of dental caries, increased risk of oral candidiasis, diminished immune response to periodontal disease, early tooth loss, and heightened proliferation of microorganisms [4, 9]. The dissemination of periodontal pathobionts and their metabolic products can lead to endotoxemia or bacteremia, which increases serum levels of inflammatory mediators such as Interleukin 6 (IL-6), fibrinogen, and C-reactive protein (CRP). Conversely, systemic inflammation can increase insulin resistance and, consequently, worsen diabetes control [8, 10].

Saliva is composed of cells, water, proteins, glycoproteins, and ions, and plays a crucial role in maintaining oral health. A significant reduction in salivary production can adversely affect oral health and diminish individuals' quality of life [11, 12]. To assess saliva's impact on oral cavity homeostasis, it is crucial to examine its components, particularly focusing on biochemical elements [13]. Salivary electrolytes such as calcium (Ca), inorganic phosphorus (Pi), and bicarbonate are essential for oral health maintenance as they contribute to oral balance, interact with proteins and cells, serve as substrates for microbiota, and participate in saliva buffering [14-16]. Furthermore, concentration of Ca and phosphate ions in saliva is critical for maintaining the balance between dental tissues demineralization and remineralization [14-16]. Dental tissues are primarily composed of hydroxyapatite minerals [$Ca_{10}(PO_4)_6(OH)_2$]. When saliva is subsaturated with Ca and phosphate, a demineralization process takes place. Conversely, saliva is known to be

supersaturated regarding these ions, constantly depositing minerals on the tooth surface, process known as remineralization [11].

More specifically, salivary Ca ions are present in various forms, primarily bound to inorganic components such as orthophosphate or carbonate, or to macromolecules such as proteins. Nearly half of the Ca in saliva exists in its ionized form, playing a crucial role in enamel remineralization as previously stated [15, 17, 18]. Interestingly, in individuals with DM, there is an increase in Ca concentration in saliva compared to normoglycemic individuals [19]. Therefore, salivary Ca levels may serve as a risk indicator of oral health, as hyperglycemic individuals levels have a higher incidence of caries compared to those normoglycemic individuals [20].

Regarding the phosphate present in either bone or teeth, approximately 85% is stored in the form of hydroxyapatite, a Ca-phosphate salt, due to the strong electronegative attraction of phosphate (Pi) to Ca ions [21]. Pi is not only important for maintaining the physical-chemical balance between dental tissues and surrounding fluids, but it is also used as a nutrient by the oral microbiota. Total saliva and parotid saliva showed high levels of Ca and Pi, which is associated with a low rate of dental caries [22, 23]. In addition, salivary phosphate levels have been associated with inflammatory indicators such as salivary C-reactive protein (CRP), and increased Pi consumption and induction of salivary inflammatory cytokines such as IL-1β, IL-4 and IL-8 have been observed [22].

Research into the diagnosis of diseases using saliva as a fluid is promising due to its simplicity, non-invasive nature and correlation with blood levels. However, only few studies have shown the differences in Ca and Pi in individuals with DM, whether in plasma or saliva. Furthermore, there is no direct correlation between the salivary alterations observed and the oral alterations caused by DM. The aim of this study was to characterize the composition of the saliva of individuals with and without glycemic control, with a specific focus on Ca and Pi. Additionally, the study aimed to investigate the potential relationship between these parameters and glycemic levels, as well as other clinical variables in adults.

3.3. MATERIALS AND METHODS

Study design and ethics

A cross-sectional study was carried out and reported using the STROBE checklist. The project was approved by the University of Brasilia School of Health Sciences ethics committee (no. 87962818.4.0000.0030). All volunteers received information about the study's aims, risks, and benefits, and signed an informed consent form.

Participants

Participants were recruited from March 2023 to December 2023 at the Diabetes clinic at the University Hospital of Brasilia. Inclusion criteria were dentated adults (≥35 years-old). Exclusion criteria comprised individuals with type 1 DM, pregnant or postpartum women (breastfeeding), transplant patients, individuals with a history of epilepsy, Sjögren's syndrome, lupus, Crohn's disease, HIV, celiac disease, radiotherapy (head and neck) or chemotherapy treatment within the last 3 months. Additionally, people with physical disabilities that prevent the measurement of height/weight and waist circumference while standing, such as cerebral palsy, paraplegia, and quadriplegia were excluded.

Variables

Sociodemographic, anthropometric, and clinical parameters were analyzed following the guidelines of the Patient-centred Outcomes for Adult Oral Health [24] and the Core Outcomes Diabetes SCORE-IT [25], which included self-reported previous diagnoses of DM.

The dataset comprised information on age, time since diagnosis of T2D, use of medication and social indicators, which were collected from the individuals at the same day of their dental clinical appointment. The level of education was registered according to the Brazilian Institute of Geography and Statistics (IBGE) for people aged 25 years or older, Brazilian Continuous National Household Sample Survey 2012-2019, as following: no education, incomplete primary education, complete primary education, incomplete secondary education, complete secondary education, and higher education.

Also on the same day, a nutritionist collected anthropometric data. The weight status was evaluated by classifying body mass index as normal weight, overweight, obesity grades 1 to 3, according DBO [26]. The same nutritionist, under the supervision of a specialist nurse, conducted capillary blood glucose measurements using a portable glucometer (Performa, Accu-Chek, and Accu-Chek Performa. "Accu-Chek®." 2019).

Fasting blood glucose (FBG) (hexokinase method; mg/dL) and glycated hemoglobin (A1c) (turbidimetric inhibition immunoassay; %) tests were carried out at the university's specialized hospital clinical laboratory (HUB/EBSERH - Brasília, Brazil). Individuals were classified according to their level of glycemic control, as normoglycemic individuals (A1c <6.5%) or hyperglycemic individuals (A1c >6.5%), as per the criteria established by the

Brazilian Society of Diabetes [27]. Saliva samples (unstimulated and stimulated) were taken on the same day to assess glycemic levels, as detailed below.

We also collected data on the use of hypoglycemic drugs, insulin and other medication that could affect salivary flow. The number of medications taken continuously by the individuals were considered, and used to classify them as “polypharmacy” (three medications or more) [28]. The number of teeth was considered all natural teeth summed with implants, and categorized as >20 teeth and 20 teeth or less.

Salivary assessments: flow rate, pH and buffer capacity

Saliva was collected in the morning (between 8-10 am) to minimize the effects of circadian rhythms. Participants were instructed to refrain from drinking, eating, and performing any physical activity for at least 8 hours before the saliva collection procedures. Unstimulated saliva was collected by allowing it to passively drooling into a universal collection tube positioned between the lower lip and chin, with the mouth slightly open. For stimulated saliva, participants were instructed to chew on a silicone device (parafilm) for 5 minutes. Saliva samples (unstimulated and stimulated) were collected for 5 minutes each. The total volume of saliva was measured and expressed in ml/minute.

Stimulated salivary pH was assessed with a laboratory pH electrode calibrated according to an appropriate standard (PHS3BW®). Salivary viscoelasticity was also measured during the transfer of unstimulated saliva to the microtube, being classified according an adaption of Gohara et al. 2004, as fluid (0cm), serous (≤ 2cm) or mucous (>2cm) [29]. The buffering capacity of the stimulated saliva sample was measured by mixing 1 ml of stimulated saliva with 3 ml of 0.005M hydrochloric acid. After 1 minute, the pH of the mixture was measured. If the saliva sample was smaller than 1 ml, the 3:1 ratio between hydrochloric acid and saliva was maintained. A pH value below 4.0 indicates low buffering capacity.

Salivary glucose concentration assay

Salivary glucose was measured from stimulated saliva using the Labtest GlicoseUV - liquiform® kit (Labtest Diagnóstica S.A - Minas Gerais, Brazil), according to the manufacturer’s instructions, and adapted for salivary volumes (150 µL of total saliva supernatant) as previously described [30]. After the incubation time, 200 µL of the reaction were transferred in duplicate to the 96-well plate, which was read on the

photometer at 505 nm. Salivary glucose was evaluated as a continuous variable in mg/dL.

Salivary calcium concentration assay

Salivary calcium was measured from stimulated saliva using the Labtest Calcio UV - liquiform® kit (Labtest Diagnóstica S.A - Minas Gerais, Brazil), was also quantified using an adapted protocol for the saliva volumes. The stimulated saliva samples were centrifuged for 1 minute and the 20 µL of supernatant added to 1000 µL of working reagent, which was prepared in a ratio of 3 volumes of reagent 1 (buffer 920 mmol/L, pH12) to 1 volume of reagent 2 (O-cresolphthaleinacomplexone 320µmol/L; 8-hydroxyquinoline 13mmol/L and hydrochloric acid 130mmol/L). A calcium standard tube was added with the same volumes (calcium 10 mg/dL). After homogenization, 200 µL of the reaction was transferred in triplicate to the 96-well plate, which was read on the spectrophotometer at 570 nm (550 ~ 590). Salivary Ca was assessed as a continuous variable in mg/dL.

Salivary phosphorus concentration assay

Salivary phosphorus was measured from stimulated saliva using an adapted protocol for the Labtest Fósforo UV - liquiform® kit (Labtest Diagnóstica S.A - Minas Gerais, Brazil). The stimulated saliva samples were centrifuged for 1 minute and the 10 µL of supernatant added to 1000 µL of reagent 1 (sulphuric acid 600 mmol/L; ammonium molybdate 2.0 mmol/L; and surfactant). A Pi standard tube was added with the same volumes (phosphorus 5.0 mg/dL). The tubes were homogenized and incubated at 37 °C for 5 minutes. After the incubation time, 200 µL of the reaction was transferred in triplicate to the 96-well plate, which was read on the spectrophotometer at 340nm. Salivary Pi was evaluated as a continuous variable. Salivary phosphorus was evaluated as a continuous variable in mg/dL.

The Labtest Kits (Labtest Diagnóstica S.A - Minas Gerais, Brazil) are systems for determining glucose, Ca and Pi by end-point reaction in blood and urine samples, but these tests have been adapted for use on saliva. A volume of 200µL of the sample was added to the reagent and then the absorbances were determined and compared with the standard at 340 ~ 700 nm, in a final volume of 200µL of the reaction in triplicate in the plate wells. All plate readings were taken by SpectraMax® microplate spectrophotometer (340PC384, SpectraMax® 190 and VersaMax™).

Sample size

A sample size calculation was performed using the OpenEpi software (version 3.0 opensource calculator - SSMean). A general sample calculation was carried out for the umbrella longitudinal study, based on 15% of outcome prevalence differences in individuals with and without T2D from a previous study [30], study power of 80%, alpha of 5%, ratio of 2:1 (two individuals without T2D for each individual with T2D) and loss rate of 30%, generating a total of 170 individuals to be examined.

Statistical analysis

Statistical analyses were carried out by comparing the averages between the groups with and without glycemic control, as well as using correlations between continuous variables. After confirming the non-parametric distribution of the data (Kolmogorov-Smirnov test), the differences between the groups were tested using the Mann-Whitney test. The chi-square and Fisher's exact tests were used to compare the distribution of the samples in the categorical variables. Spearman's correlations were used to correlate salivary glucose levels with salivary Pi and salivary Ca in all sample, as well as in subsamples of normoglycemic individuals (A1c<6.5%) and the ones with self-reported no diagnosis of T2D. The significance level was set at 5%. The data was analyzed using SPSS version 26.0.

3.4. RESULTS

Characteristics of the sample

A total of 187 individuals were assessed, 17 of whom were excluded from the study because they did not meet the inclusion criteria (Figure 1). The final sample consisted of 170 individuals (mean age 54.3 ± 10.9 years). Of these, 95 had a diagnosis of T2D (33 men and 62 women) and 75 had no previous diagnosis of T2D (28 men and 47 women). As for glycemic control, 157 individuals were assessed (mean age 54.5±11 years). Of these, 55 had an A1c level ≥ 6.5% (24 men and 31 women) and 102 had an A1c level < 6.5% (33 men and 69 women).

Table 1 summarizes the general characteristics of the participants with and without glycemic control according to sociodemographic, systemic health, oral health, and salivary data. The majority of the sample was female (63.4%), although there was no significant difference between the groups (p=0.11). An overall mean age of 54.55±11.09 years was found (p= 0.13). There was also no significant difference between educational level and glycemic status (p= 0.17). As expected, individuals in uncontrolled hyperglycemia had more diagnosis of T2D, years with a diagnosis of T2D, number of

medications administered daily and polypharmacy, and higher capillary blood glucose, CRP, blood FBG and A1c than normoglycemics. There was also a significant association of glycemic status and smoking habits (p = 0.04), but surprisingly there was no significant associations with obesity (p= 0.18), probably because more than 76.8% of the sample had overweight and obesity. Regarding oral health status, number of teeth (natural and implants), bleeding on probing, and the use of partial removable prostheses were similar between normoglycemic and hyperglycemic groups.

Table 1 also illustrates the results for all salivary analyses, including salivary flow (both resting and stimulated), salivary pH, salivary buffering capacity and viscoelasticity. No significant differences were observed between the groups for any of these variables. Individuals in hyperglycemia exhibited significantly elevated levels of salivary Ca (p=0.02) than normoglycemics, with mean values of 11.09 ± 6.52 mg/dl. Unexpectedly, individuals in normoglycemia demonstrated significantly elevated levels of salivary glucose (p=0.04), with mean values of 31.99±15.38. No significant differences were observed in salivary Pi levels between the groups (p=0.18).

Averages of Ca and Pi levels according to the participants' characteristics is shown in Table 2. No statistically significant difference was observed between salivary Ca and Pi levels for sex (p = 0.77), diagnosis of T2D (p=0.52), salivary viscoelasticity (p=0.46), the use of multiple medications, weight status, and the number of teeth (>20 teeth vs. <20 teeth).

Spearman's correlation of Ca and Pi is shown in table 3. When the complete sample was considered, there was a weak positive correlation between salivary Ca with years with diagnosis of DM (Rho= 0.2; p= 0.015), FBG (Rho= 0.187; p= 0.026), and A1c (Rho= 0.208; p= 0.016). However, there were no significant correlations with other factors such as age, years with T2D, number of teeth, salivary and stimulated flow, salivary pH, buffer capacity, CRP, number of medications and capillary glycaemia.
Interestingly, salivary glucose was negatively correlated with Ca (Rho=-0.42; p=0.000), and positively correlated with Pi (Rho= 0.26, p=0.002) (Figure 2). It seems that this was not influenced by the salivary flow (Figure 3). To mitigate the impact of hyperglycemia on these correlations, we conducted the analysis using a subsample of normoglycemic controls (A1c<6.5%). The significant correlation between salivary glucose with both Ca and Pi persisted. In this subsample, however, the higher Pi level the lower the number of teeth (Rho= -0.230; 0.029). We also tested these correlations exclusively in individuals without a diagnosis of DM and the significant correlation between salivary glucose and Ca still persisted (Rho= -0.486; p=0.000), whereas this was not observed for Pi.

3.5. DISCUSSION

In the present study, glucose, Ca and Pi were assessed as salivary parameters aiming to investigate the influence of glycemic factors on the maintenance of oral biochemical balance. We confirmed that Ca levels increase with poor glycemic control (FBG and A1c), indicating saliva's potential as a biomarker for Ca measurements in this context. Conversely, salivary glucose levels showed an inverse relationship with salivary Ca: higher salivary glucose correlated with lower Ca levels. Additionally, Pi levels were positively associated with salivary glucose levels.

Persistent hyperglycemia is a risk factor for oral diseases such as hyposalivation and dental caries [9, 20, 31, 32]. It has been described that the incidence of root and coronal caries is higher in T2D than in healthy individuals [20]. Many studies have been carried out to investigate the biochemical changes that could be involved in this association [33-38], including variations in the concentrations of glucose, total protein, electrolytes such as Pi and Ca, and buffering capacity [7]. Using a colorimetric kit adapted for saliva, it was revealed that salivary glucose levels were lower in individuals with A1c ≥ 6.5% compared to those with A1c < 6.5% in our sample (Table 1). This finding contrasts with our previous results from another sample using the same kit, where salivary glucose correlated with A1c and FBG [30]. This discrepancy may stem from differences in sampling employed in the respective studies. In the present study, it was recommended that participants undergo blood and saliva analysis on the same day, necessitating a fasting period of at least 8 hours, which is quite lengthy for saliva assessment. Due to the continuous use of antidiabetic medication and insulin, the individuals may have been in a hypoglycemic state during saliva collection, which is a confounding factor in the final salivary glycaemia assessment. Meanwhile, in our previous study, participants were only required to fast for 2 hours before saliva collection. Despite saliva and blood samples being collected on different days, salivary glucose still mirrored systemic glycemia [30].

Salivary Ca and Pi were successfully assessed in the present sample. Salivary Pi concentration was similar in normoglycemic and hyperglycemic individuals (Table 2). As discussed before, Pi is known to play a crucial role in saliva buffering and dental remineralization [16], with its concentration best assessed in whole stimulated saliva, reflecting its level in dental plaque. Studies have indicated that Pi has a preventive effect against dental caries [39]. In contrast to the findings of Ladgotra et al. [33] and Ambikathanaya et al. [16], both studies showed higher mean Pi values in the DM groups compared to the normoglycemic control groups. However, our findings were similar to

the study by Tiongco et al. [35], which also did not show a significant increase or association in salivary Pi levels between diabetic and non-diabetic patients.

To our knowledge, this study marks the first observation of a positive correlation between salivary glucose and salivary Pi concentrations, suggesting that higher glucose levels coincide with elevated Pi levels. This was maintained when only normoglycemic individuals were analyzed. Further research is needed to explore how this relationship may impact oral health or reflect systemic conditions. Our findings did not demonstrate any significant effects of variables such as sex, duration since diagnosis of DM, saliva viscosity, polypharmacy, weight status, or number of teeth on Pi levels.

Higher levels of Ca were observed in individuals with poor glycemic control (Table 1). These results are consistent with those of Ladgotra et al. [33] and Mrag et al. [36] who found a significant difference in salivary Ca levels between groups with and without DM. According to Ladgotra et al. [33], the increase in salivary Ca in patients with DM may be due to a decrease in salivary flow or to the presence of specific proteins that bind to calcium phosphate complexes. High levels of Ca in saliva serve as a positive indicator for oral health. In contrast, a negative correlation was observed between salivary glucose and salivary Ca concentrations, despite the exclusion of hyperglycemic or diabetic individuals (Table 3). This is potentially related to the hypoglycemic state among participants, as previously discussed. Since insulin facilitates glucose transport by increasing insulin receptor expression, it enhances insulin responsiveness in glucose transport or indirectly regulates extracellular cytoplasmic Ca levels [35].

It is worth noting that in our sample, there were no significant differences in salivary flow based on glycemic status, and salivary flow was not correlated with Ca and Pi levels. This indicates that variations in Ca and Pi may be independent of salivary flow and are not influenced by the circadian rhythm, which does not alter the biochemical components. Therefore, saliva may serve as an alternative biological fluid for diagnostic purposes, particularly for Ca. Our test accurately differentiated between hyperglycemic and normoglycemic groups, underscoring saliva's potential utility in biochemical assessments.

Changes in the inorganic composition of saliva during hyperglycemia, such as Pi levels, can potentially impair its protective mechanisms, increasing the risk of ionic imbalance in the oral cavity. Both salivary Ca and salivary Pi play a crucial role in enamel remineralization, as evidenced by their saturation levels [14]. However, it is known that salivary Ca and phosphate are positively correlated in healthy individuals [35], which was not observed in this study. This indicates that DM status and metabolic control may

influence the long-term progression of dental caries, based on the hypothesis that elevated A1c levels are associated with greater oral imbalance [30].

This study presents an intriguing finding by conducting a correlation between salivary components, a novel approach that has not been previously investigated. Strengths of the study include a suitable, calculated sample size. Furthermore, A1c was used to assess glycemic status of individuals, not relying on self-report diagnosis of T2D. Finally, the colorimetry tests (measured in mg/dL) were conducted using affordable national kits originally designed for analyzing glucose, Ca, and Pi in blood and urine, and were successfully adapted for use with saliva samples. Since most laboratory kits need to be imported for research and clinical use in the Global South, having a locally produced kit makes the process much more cost-effective. On average, it costs $0.15 per sample analyzed, making it significantly more affordable compared to other available options on the market. This innovative approach has the potential to make salivary diagnosis accessible for all world populations.

As of study limitation, participants attended appointments on-demand, leading to weak external validity. Additionally, 13 individuals did not have sufficient saliva for assays, although this did not impact the statistical analysis.

3.6. CONCLUSION

In conclusion alterations in the inorganic composition of hyperglycemic saliva, particularly in Ca levels, may impair its protective mechanisms, leading to an increased risk of ionic imbalance and, consequently, an increased incidence of caries development. Saliva has the potential to be a practical and accessible tool for monitoring systemic conditions, and in individuals with DM could be useful as an oral health risk assessment.

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TABLES AND FIGURES CAPTION

Table 1: Sociodemographic, systemic health, oral health, and salivary characteristics of adult individuals categorized by the glycaemic control (A1c < 6.5% vs. A1c ≥ 6.5%).

	A1c < 6.5 (N= 102)		A1c ≥ 6.5 (N= 55)		P*
	N(%)	Average±SD	N(%)	Average±SD	
Age		53.49±11.14		56.47±10.18	0.13
Sex					0.11
Male	33 (21%)		24 (15.3%)		
Female	69 (44%)		31 (19.7%)		
Educational level					0.17
No education	1 (0.98%)		1 (1.8%)		
Incomplete primary education	23 (22,6%)		10 (18.2%)		
Complete primary education	5 (4.9%)		6 (10.9%)		
Incomplete secondary education	6 (5.9%)		9 (16.4%)		
Complete secondary education	34 (33.4%)		13 (23.7%)		
Higher education	33 (32.4%)		16 (29%)		
Year with diagnosed T2D		1.7±3.86		11.05±7.25	**0.00**
Number of medications administered daily		1.91±2.43		4.94±2.87	**0.00**
Polypharmacy					**0.00**
No	70(46.7%)		9(6%)		
Yes	27(18%)		44(29.3%)		
Smoke					**0.04**
No	69(44%)		32(20.4%)		
No, but used to smoke	22(14%)		21(13.3%)		
Yes	11(7%)		2(1.3%)		
Weight status					0.18
Normal weight	29(18.7%)		7(4.5%)		
Overweight	40(25.8%)		21(13.6%)		
Obesity grade 1	23(14.8%)		16(10.3%)		
Obesity grade 2	6(3.9%)		4(2.6%)		
Obesity grade 3	4(2.6%)		5(3.2%)		
Capillary blood glucose		98.88±16.07		160.58± 61.36	**0.00**

C-reactive protein		0.40±0.53	0.51±0.51	**0.03**
Blood FBG (ml/dl)		100.50±13.35	172.07±71.05	**0.00**
Blood A1c (%)		5.64±0.52	8.60±1.69	**0.00**
Number of teeth (natural + implants)		22.38±7.03	20.93±6.88	0.13
Bleeding on Probing (mean proportion)		0.5±1.78	0.97±4.20	0.26
Partial removable prothesis				0.22
None	72(45.8%)		33(21%)	
Partial - Upper	11(7%)		11(7%)	
Partial - Upper and Lower	13(8.4%)		9(5.7%)	
Complete - Upper	4(2.5%)		0(0%)	
Partial - Lower	2(1.3%)		2(1.3%)	
Saliva flow rest		0.29±0.32	0.25±0.28	0.34
Saliva flow stimulated		0.35±0.30	0.35±0.32	0.81
Saliva pH		7.33±0.58	7.49±0.71	0.16
Saliva buffer		3.77 ±1.20	4.07±1.46	0.31
Saliva viscosity				0.09
Fluid	37 (25.5%)		22 (15.2%)	
Serous	9 (6.2%)		9 (6.2%)	
Viscous	51(35.2%)		17 (11.7%)	
Saliva glucose (ml/dl)		31.99±15.38	26.88±18.80	**0.04**
Saliva Calcium (ml/dl)		8.71±6.12	11.09±6.52	**0.02**
Saliva Phosporous (ml/dl)		7.17±2.07	6.56±1.13	0.18

*Bold p-values means significance, Chi-Square or Mann-Whitney tests; A1c = glycate hemoglobin; FBG = Fasting blood glucose. SD=standard deviation.

Table 2: Averages of Calcium (Ca) and Phosphorous (Pi) according to the characteristics of participants. N= 157.

	Ca		Pi	
	Average±SD	**P**	**Average±SD**	**P**
Sex		0.21		0.77
Female	8.86±5.91		7.01±2.07	
Male	10.29±6.91		6.84±1.19	
Year with diagnosis of DM		0.11		0.52
No DM	65.21		75.77	
1 year or less	59.71		60.64	
2-9 years	81.14		66.11	
10 years or more	81.73		76.22	
Saliva viscosity		0.30		0.46
Fluid	10.12±6.82		6.98±1.37	
Serous	7.56±5.16		6.66±2.12	
Viscous	9.21±6.09		6.94±1.8	
Polypharmacy		0.358		0.784
No	9.19±6.55		6.98±2.03	
Yes	9.84±6.29		6.90±1.56	
Weight status		0.343		0.620
Normal weight	11.21±6.86		7.08±1.69	
Overweight	9.05±6.69		7.14±2.18	
Obesity grade 1	8.34±5.78		6.71±1.50	
Obesity grade 2	8.71±5.92		6.61±1.29	
Obesity grade 3	9.54±3.43		6.33±0.95	
Number of teeth		0.709		0.212
20 or less	9.28±6.93		7.07±1.52	
More than 20	9.14±5.98		6.88±1.93	

*Mann-Whitney or Kruskal-Wallis Tests; SD=standard deviation; med (min-max)

Table 3. Spearman correlation of the salivary Calcium (Ca) and Phosphorous (Pi) and characteristics of individuals (age, years with diagnosis of type 2 Diabetes Mellitus, number of natural teeth and implant, saliva flow rest and stimulated, saliva pH, saliva buffer, salivary glucose, bleeding on probing, glycated hemoglobin-A1c%, fasting blood glucose-FBG, C-reactive protein, number of medications administered daily, and capillary blood glucose.

		All sample*		**Only individuals with good glycaemic control (A1c<6.5%)**		**Only individuals without Diagnosis of DM**	
		Ca	Pi	Ca	Pi	Ca	Pi
Age	Rho	-0.046	0.140	-0.173	0.160	-0.130	0.200
	P	0.588	0.093	0.109	0.132	0.297	0.099
	N	144	146	87	90	66	69
Years with DM	Rho	**0.202**	-0.014	0.050	0.042	-0.114	0.049
	P	**0.015**	0.868	0.647	0.692	0.362	0.691
	N	144	146	87	90	66	69
Number of natural teeth + implants	rho	-0.074	-0.134	0.031	**-0.230**	-0.036	-0.133
	P	0.378	0.106	0.776	**0.029**	0.773	0.277
	N	144	146	87	90	66	69
Saliva flow rest	Rho	0.017	-0.068	0.043	-0.158	0.003	-0.202
	P	0.838	0.412	0.692	0.136	0.978	0.096
	N	144	146	87	90	66	69
Saliva flow stimulated	Rho	0.135	-0.024	0.152	-0.141	0.149	-0.207
	P	0.106	0.774	0.161	0.184	0.232	0.087
	N	144	146	87	90	66	69
Saliva pH	Rho	-0.026	-0.102	-0.055	-0.056	-0.122	-0.052
	P	0.766	0.242	0.635	0.617	0.348	0.683
	N	131	133	78	81	61	64
Saliva buffer	Rho	-0.068	-0.006	0.000	0.040	0.077	-0.019
	P	0.441	0.941	0.997	0.724	0.558	0.881
	N	131	133	78	81	61	64
Saliva glucose	Rho	**-0.423**	**0.259**	**-0.434**	**0.229**	**-0.486**	0.226
	P	**0.000**	**0.002**	**0.000**	**0.032**	**0.000**	0.061
	N	140	144	84	88	65	69
Bleeding on probing (average %)	Rho	0.007	0.080	-0.123	0.116	-0.084	0.144
	P	0.933	0.340	0.255	0.275	0.504	0.239
	N	144	146	87	90	66	69
Blood FBG (ml/dl)	Rho	**0.187**	-0.028	0.139	0.130	-0.071	0.166
	P	**0.026**	0.736	0.200	0.224	0.570	0.173
	N	142	144	87	90	66	69
Blood A1c (%)	Rho	**0.208**	0.002	0.046	0.140	0.009	0.112
	P	**0.016**	0.978	0.674	0.188	0.948	0.381
	N	133	135	87	90	60	63
C-reactive protein mg/dL	Rho	0.067	-0.096	0.130	-0.013	0.078	0.090

	P	0.434	0.257	0.231	0.902	0.535	0.460
	N	140	142	87	90	66	69
Number of medications administered daily	Rho	0.046	0.016	-0.013	0.101	-0.076	0.064
	P	0.597	0.849	0.904	0.357	0.543	0.604
	N	137	140	82	86	66	69
Capillary blood glucose	Rho	0.159	-0.037	-0.049	0.082	-0.130	0.200
	P	0.057	0.663	0.650	0.442	0.297	0.099
	N	143	145	87	90	66	69

* Missing data in the total sample indicates that there was insufficient saliva to conduct the assays. Rho= Spearnans' correlation coefficient.

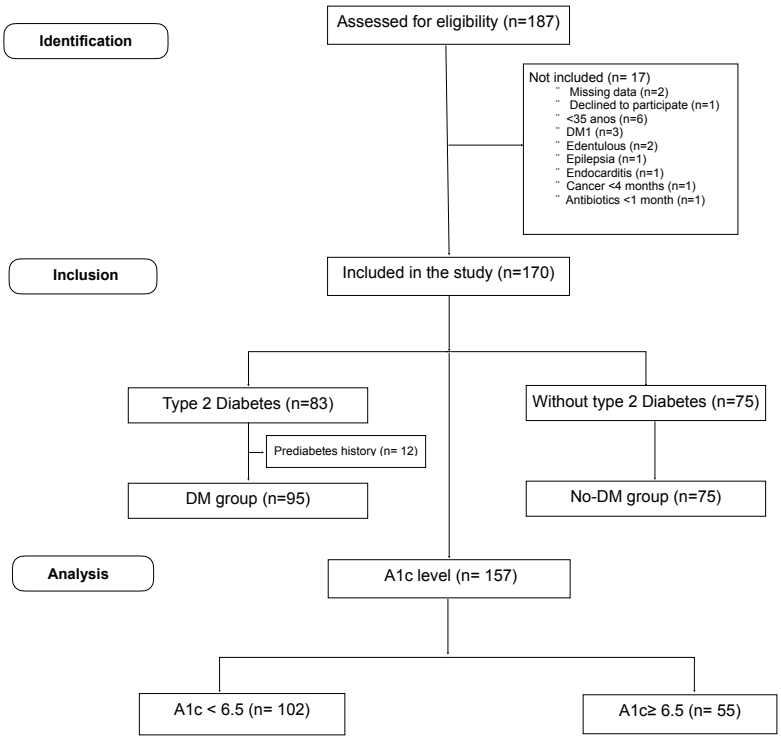

Figure 1. STROBE flowchart - From the total sample, 170 individuals were evaluated, with 95 individuals having type 2 diabetes mellitus (T2D) and 75 without T2D. Among them, 102 individuals had glycate hemoglobin (A1c) < 6.5, whereas 55 individuals had A1c ≥ 6.5.

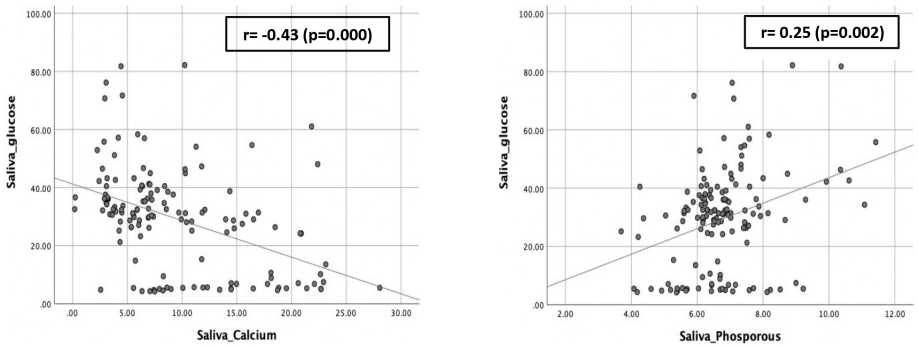

Figure 2. Correlation between salivary glucose levels and calcium (Ca) and phosphorus (Pi) concentrations, as determined by the Spearman correlation test.

4. CAPÍTULO 4- DISCUSSÃO GERAL E CONCLUSÕES DA DISSERTAÇÃO

4.1. DISCUSSÃO GERAL

A saliva desempenha um papel crucial na saúde bucal e na homeostase, contribuindo significativamente para as funções de defesa do hospedeiro [1]. Além disso, a saliva é considerado um excelente material diagnóstico, pois as alterações nos seus componentes salivares podem ser reflexo de mudanças sofridas na composição sanguínea [2]. Além disso, a coleta desse fluido pode ser realizada de forma fácil e não invasiva [3-7].

Discutimos nessa dissertação que a sialometria é método clínico mais recomendado para quantificação do fluxo salivar total não estimulados e estimulados. Em nossa meta-análise, o fluxo salivar não estimulado foi de 0,82 mL/min (IC = 0,66-0,99; P = 0,0005), enquanto o fluxo salivar estimulado foi de 1,44 mL/min (IC = 1,26-1,61; P = 0,005). Esses resultados demonstram que, para uma avaliação precisa, é crucial reconsiderar os valores de referência para diagnóstico de hipossalivação. Isso pode estar gerando resultados falso-negativos na identificação de problemas iniciais de volume salivar e potencialmente influenciar na relação xerostomia vs. hipossalivação. Além disso, intervenções equivocadas ou a falta de intervenção pode estar associada com esse diagnóstico falho, pois as variações no fluxo salivar de indivíduos saudáveis podem diferir dos padrões comumente utilizados na prática clínica e na pesquisa odontológica.

Na revisão sistemática apresentada no capítulo 2 dessa dissertação, observou-se que, em muitos estudos, o fluido salivar é equiparado à densidade da água, considerando que 1 g = 1 ml de saliva. A quantidade de saliva em gramas foi convertida em mililitros, assumindo que a densidade da saliva é de 1 g/mL. Essa equivalência permite registrar o peso da saliva para fins de avaliação da taxa de fluxo salivar não estimulada em ml/min [8-11]. Isso evidencia que, além das discrepâncias nos pontos de corte, existem diferenças substanciais na metodologia de avaliação da saliva, deixando evidenciado o desafio em definir parâmetros consensuais para a caracterização da hipossalivação.

Com a presença de diversos componentes sanguíneos na saliva, os avanços em biotecnologia têm possibilitado a obtenção de diversos diagnósticos relacionados a sistemas fisiológicos [12]. Assim, a saliva é cada vez mais utilizada e validada como um biofluido para diagnóstico, monitoramento e predição de doenças. A complexidade deste fluido oral é melhor compreendida ao se considerar seus diversos componentes e funções [13]. Porém, nota-se que para DM isso não é tao verdade. Um dos principais achados deste estudo foi sobre o protocolo de coleta da saliva para realizar o teste de

glicose. Na maioria dos estudos, os indivíduos são orientados a não ingerir líquidos, a não se alimentar e a não realizar atividade física pelo menos duas horas antes dos procedimentos de coleta salivar. No entanto, neste estudo, os indivíduos estavam em jejum superior a 8 horas, visto que no mesmo dia da coleta de saliva, os indivíduos realizavam exames de sangue (avaliação da glicemia em jejum) e avaliação de glicemia capilar antes da coleta de saliva. Esse jejum prolongado pode ter impactado a avaliação da saliva. Além disso, o uso contínuo de medicação antidiabética e insulina pode ter induzido um estado hipoglicêmico durante a coleta, o que pode constituir um fator de confusão na avaliação final da glicemia salivar. Entretanto, em nosso estudo anterior, os participantes apenas tiveram que estar em jejum durante 2 horas antes da coleta de saliva. Apesar de as amostras de saliva e sangue terem sido colhidas em dias diferentes, a glicemia salivar ainda refletia a glicemia sistêmica utilizando esse kit nacional e com bom custo-benefício [14]. Por esse motivo, acreditamos que um diagnóstico de DM baseado na saliva, com bom custo-benefício, está muito longe de chegar ao paciente e superar a precisão da glicemia capilar.

Mas apesar da glicose salivar ter sido extremamente influenciada pelo jejum, outras alterações nos componentes salivares podem refletir mudanças na composição sanguínea [2, 15]. Entre os eletrólitos salivares, Ca e Pi tem importância para a saúde bucal, contribuindo para o equilíbrio entre dissolução e remineralização do esmalte dental [16-18], mas também são fundamentais para a saúde do sistema esquelético humano [19]. Altas concentrações de Ca e Pi na saliva total e parótida estão associadas a uma menor taxa de cárie dentária [5, 20]. Aqui, observamos que Ca e Pi apresentaram associados com a glicose salivar. Sendo uma correlação negativamente entre glicose e o Ca (Rho= -0,42; p=0,000) e uma correlação positivamente entre glicose e oPi (Rho= 0,26, p=0,002). O fluxo salivar, no estado normoglicémico ou no diagnóstico de DM não tiveram um impacto significativo nesta correlação.

Além disso, no contexto da DM2, é comum que os pacientes apresentem condições associadas como hipertensão arterial e comprometimentos cardiovasculares [13]. A hipertensão arterial é um fator de risco significativo para DM2, pois a pressão arterial elevada e a atividade aumentada do sistema nervoso reduzem a captação de glicose, levando à resistência à insulina [13]. Essa associação, em geral, leva os pacientes a estarem em polifarmácia, com o uso de medicamentos como anti-hipertensivos e diversos medicamentos anti-diabetes. Isso pode induzir disfunções nas glândulas salivares e influenciar as concentracoes de glicose na saliva. No nosso estudo transversal (capítulo 4), a polifarmácia foi associada com descontrole glicêmico. O próximo passo nesta linha de pesquisa é investigar como o uso de medicamentos,

incluindo quais classes específicas, está influenciando a quantidade e a qualidade da saliva em pacientes. O nível de controle glicêmico também influencia a condição bucal; um estudo mostrou uma tendência de diminuição do fluxo salivar com o aumento dos níveis de HbA1c, o que está relacionado a riscos de complicações microvasculares e cardiovasculares [21]. A inflamação geral associada ao descontrole glicêmico pode resultar em maior expressão de mediadores inflamatórios, afetando negativamente a resposta imunológica [21, 22]. Evidências sugerem que o controle metabólico sistêmico pode melhorar com a terapia periodontal e a redução da inflamação nos tecidos bucais [23]. Ao utilizar dois marcadores inflamatórios nas nossas análises (proteína C-reativa e sangramento a sondagem), observamos mais inflamação nos pacientes em estado de hiperglicemia (A1c >6,5%), porém esses fatores não foram correlacionados com níveis salivares de Ca e Pi.

Portanto, devido à sua composição complexa e única, a saliva é fundamental para avaliar a homeostase oral e a saúde geral. A análise dos componentes salivares, especialmente os bioquímicos, pode oferecer uma ferramenta valiosa para monitorar estados fisiológicos, uma vez que sua concentração é menos influenciada por fatores circadianos em comparação com a glicose. Assim, a saliva se destaca como uma ferramenta promissora para o monitoramento do DM.

4.2. CONCLUSÃO

Os avanços nas tecnologias de coleta e análise de saliva, combinados com a capacidade de detectar alterações sistêmicas, destacam a importância de revisar e atualizar os parâmetros padrão de fluxo salivar e metodologias de coleta em saúde. Além disso, é fundamental desenvolver novos parâmetros baseados em evidências para o diagnóstico da hipossalivação. Observa-se que a média do fluxo salivar em indivíduos adultos saudáveis é substancialmente superior ao ponto de corte atualmente empregado para a definição da condição. Assim, o ponto de corte estabelecido pode ser insuficientemente baixo, o que pode levar a uma subavaliação da hipossalivação. A relação entre os níveis de componentes salivares, como por exemplo Ca e Pi, e a saúde sistêmica (como os parâmetros de A1c) reforça a necessidade de uma abordagem integrada para o cuidado em saúde e potencial da saliva nessa abordagem. A capacidade da saliva de refletir mudanças na composição sanguínea e sua relevância na triagem de grandes populações sublinha seu potencial como ferramenta diagnóstica. Portanto, mais estudos são necessários para o desenvolvimento na área para aprimorar a precisão e a aplicabilidade clínica do diagnóstico salivar.

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5. CAPÍTULO 5 – PRESS RELEASE

A saliva é muito importante para saúde bucal. Mas, infelizmente, só costuma ser notada em sua ausência total, no caso de doenças que afetam as glândulas salivares. Na ausência de saliva, podem ocorrer xerostomia (boca seca), dificuldades na fala e na deglutição, além de aumentar o risco de cárie e infecções orais. Porém, outras situações também diminuem o fluxo salivar em menor grau (hipossalivação), as vezes despercebido pelas pessoas, apesar de já causar diversas complicações. Portanto, saber quais os métodos de medição do fluxo salivar seriam associados a saúde é de grande valia para o dentista atuar de forma preventiva à essas complicações. Em nosso estudo, calculamos uma média os valores do fluxo salivar em pessoas saudáveis, obtendo valores médios de 0,8 ml/min para saliva não estimulada e 1,45 ml/min para saliva estimulada. Ao comparar com indivíduos com alterações diversas causadoras de hipossalivação, observamos que o fluxo salivar não estimulado e estimulado é, em média, 0,4 ml/min menor do que em pessoas saudáveis. O diabetes mellitus tipo 2 é um dos agravos que pode reduzir a capacidade de produção de saliva. Por isso, nessa dissertação, também analisamos parâmetros qualitativos da saliva, como glicose, cálcio (Ca) e fosfato (Pi) em pessoas com e sem diabetes. Os níveis desses componentes na saliva têm um impacto significativo na saúde, destacando a importância de uma abordagem integrada para monitorar a saúde bucal e geral. É fundamental continuar investindo em pesquisa e desenvolvimento na caracterização dos componentes salivares para melhorar a precisão e a aplicabilidade desses métodos na prática clínica, tornando-os mais eficazes e acessíveis para avaliar a saúde e também para determinar necessidade de tratamento para problemas como hipossalivação.