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SALIVA-based diagnostic approach for diabetes mellitus: a step towards non-invasive detection – a scoping review

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Abstract. – OBJECTIVE: Diabetes mellitus (DM) is a chronic autoimmune disease whose main feature is chronic hyperglycemia. The causes of DM are impaired insulin secretion, impaired insulin action, or both. Saliva is a biofluid that can be considered as a "mirror" reflecting our body's health status; with the rapid advancement in salivaomics, saliva, being a non-invasive and safe source, could be a substitute for blood in the diagnosis and prognosis of diseases. As there are no precise guidelines about the salivary biomarkers correlated with the diagnosis of diabetes, a review was conducted to verify whether saliva analysis can be feasible and which biomarkers are more reliable, for the diagnosis of this disease.

MATERIALS AND METHODS: A literature search was performed through PubMed, Medline, Scopus, Web of Science, LILACS, Open Grey, and Cochrane Library databases. The "PRISMA" guidelines were used for the following review, and thirty-three studies were analyzed.

RESULTS: Almost all salivary glucose studies suggest that the estimation of this biomarker can be used as a potential indicator. Furthermore, studies that considered other biomarkers such as 1,5-anhydroglucitol, alpha-amylase, N-acetyl-β-D-hexosaminidase, asprosin, resistin, and fructosamine reported that these biomarkers resulted to be potentially useful for diabetes screening and diagnosis, with the exception of the cystatin SA.

CONCLUSIONS: In conclusion, several salivary biomarkers could be useful for monitoring DM, but it would be necessary to further expand the research and define precise values for each marker in order to predict with reasonable confidence if an individual is healthy or suffering from diabetes. Finally, standardized saliva collection and processing techniques are key to minimizing interindividual variability in saliva composition.

Key Words:
Diabetes mellitus, Saliva, Diagnostic, Saliva-based diagnostic.

Introduction

Diabetes mellitus (DM) is a general term for "heterogeneous disorders of metabolism whose main feature is chronic hyperglycemia". The causes of DM are impaired insulin secretion, impaired insulin action, or both[1].

The most frequent form of diabetes mellitus (DM) is the Type 2 variant (T2DM), which mostly affects adults and the elderly. However, an alarming increase of children being afflicted by this condition has been reported also correlated to the obesity epidemic. T2DM is characterized by insulin resistance, hyperglycemia, and, eventually, a dysfunction of the insulin-producing cells. The most severe forms of DM are generally linked to the Type 1 variant (T1DM), which can affect any age group but typically affects children and adolescents, with a peak of incidence around 12-13 years of age. In these cases, total or near-total destruction of the insulin-producing cells (the β-cells contained in the pancreatic islets of Langerhans) occurs[2,3].

DM is a metabolic disease with a high prevalence worldwide, representing an important global public health problem. It is estimated that by 2025, about 10% of the world's population, or approximately 425 million individuals, will be affected by diabetes. Of these, 90% suffer from type 2 diabetes. The acute and chronic complications of diabetes, such as nephropathy, retinopathy, cardiovascular diseases, or diabetic foot, have been associated with hospitalization and may be a cause of mortality[4-6].

To date, urine and blood tests are widely and readily available for screening, diagnosis, prognosis, and monitoring across the globe. However, the estimates of glucose levels using urine and blood samples are associated with several challenges. For instance, urine and glucose

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positivity cannot be detected in the early stages but at an advanced stage (of DM). On the other hand, venous blood sampling is more invasive than capillary blood sampling, thus making patients feel uncomfortable. There is a need to use a more convenient and comfortable sampling procedure that is non-invasive, reliable, and requires less expertise[7-9].

The American Diabetes Association (ADA) has established diagnostic thresholds of blood glucose levels to be evaluated by measuring blood glucose after fasting for 8 h. Patients are classified into having diabetes for blood glucose levels ≥ 126 mg/dL, pre-diabetes for levels between 100 and 125 mg/dL, and being normal for levels < 100 mg/dL. Another diagnostic method is the non-fasting blood glucose test performed after a meal, in which normal blood glucose levels fall < 200 mg/dL, with normal long-term levels returning at < 100 mg/dL after 3 h[1].

Saliva is a biofluid that can be considered as a "mirror" reflecting our body's health status[10-13]. It is a hypotonic solution of salivary acini, gingival crevicular fluid, and oral mucosal exudates. Approximately 90% of saliva is secreted from the salivary glands and the major glands include the parotid glands, submandibular glands, and sublingual glands. The salivary glands with high permeability are surrounded by abundant capillaries, blood, and acini, and can exchange molecules. Thus, biomarkers in the blood circulation can infiltrate acini and, eventually, be secreted into the saliva. It has multiple functions, including mouth cleaning and protection, antibacterial effects, and digestion. In the past, doctors have diagnosed diseases with the use of serum or urine tests, which are either painful or embarrassing. However, saliva is now considered a potential pool of biological markers that range from changes in biochemicals, DNA, RNA, and proteins to the microbiota structure. It is relatively safe to collect saliva, and it minimizes the risk of a virus being spread. With the rapid advancement in salivaomics, saliva, being a non-invasive and safe source, could be a substitute for blood in the diagnosis and prognosis of diseases such as dental caries and periodontal disease, as well as cancer, diabetes, and other systemic disorders[14-16]. For example, we are all aware of the importance that salivary diagnosis has assumed during the COVID-19 pandemic. As there are no precise guidelines about the salivary biomarkers correlated with the diagnosis of diabetes, a review was conducted to verify whether saliva analysis can be feasible and which biomarkers are more reliable for the diagnosis of this disease.

Materials and Methods

The protocol of this scoping review was registered in Open Science Framework (OSF). A search was carried out on PubMed, Medline, Scopus, Web of Science, LILACS, Open Grey, and Cochrane Library databases. The latest electronic search was carried out on February 11, 2023.

The search string used on PubMed included:

- "Diabetes Diagnosis" and "Saliva"
- "Salivary Glucose Values" and "Diabetes"

The filters applied were:

- Studies published in the last 10 years
- Articles in English

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were used for the following review.

The articles retrieved with the search strategy were entered into EndnoteWeb to eliminate duplicates.

The first screening was made by reading the titles. Excluded from consideration were studies that did not align closely with the topic; numerous articles concentrating on the connection between diabetes and periodontitis were thus omitted. If the title was not clear enough to determine whether to include or exclude the article, the abstract was read. All eligible studies underwent full-text assessment based on specific selection criteria, to determine the final list of included studies. The studies excluded at this stage were listed, specifying the reason for exclusion.

The inclusion criteria applied were:

- Comparative studies, e.g. randomized controlled trials, cohort studies, case-control studies, cross-sectional studies.
- Studies focused on salivary biomarkers useful for the diagnosis of type I and type II diabetes mellitus.

The exclusion criteria were:

- Reviews, systematic reviews, meta-analyses, and preclinical studies.
- Articles that were too general, focusing on saliva as a general method of diagnosing various diseases.
- Articles regarding gestational diabetes.

Two reviewers independently selected the articles, and then a third reviewer checked and confirmed the selection. From the included studies, two authors extracted the most relevant information and data, especially regarding the features of

the subjects, the type of markers, the method for measurement, and the main results.

Results

Of the initial list of 484 articles, after the first screening, 54 were considered eligible and underwent full-text assessment. In the end, 33 articles[17-49] were selected through the selection process represented in Figure 1.

Study Characteristics

The main features of the included studies are summarized in **Supplementary Table I**. Most of the articles were carried out in India, while the remaining 20 in other countries of the world.

In all articles, the absence of conflicts of interest was declared, and most of the studies were not funded, except for 6 articles[25,29,30,35,41,43] which were funded by public bodies, and the study by Hegde et al[45] which was funded by Colgate-Palmolive (India).

Almost all studies are case-control, with the exception of the articles by Ganesan et al[24] and by Kandavel and Kumar[50], which are cross-sectional studies, and the study by Egboh et al[51], which is a randomized trial.

Glucose

Many studies[17-29,44] were focused on the usefulness of salivary glucose as a marker for the diagnosis of diabetes mellitus. A total of 1,758 patients were analyzed, and almost all studies suggest that salivary glucose level estimation can be used as a potential indicator in the screening, diagnosis and monitoring of DM, with the exception of the Deepa Lakshmi et al[28] and Gupta et al[27] studies which agree that salivary glucose cannot replace standard blood glucose estimation methods in diabetic patients.

Other Markers

Other studies selected in the review were focused on different markers; 2 studies[30-31] considered 1,5-anhydroglucitol as a marker for a total number of 1,010 patients, one study[32] focused on salivary alpha-amylase and involved 80 patients, 1 study[33] on N-acetyl-β-D-hexosaminidase and considered 140 patients, 1 study[34] on asprosin with a total of 60 patients, 1 on cystatin SA[35] and

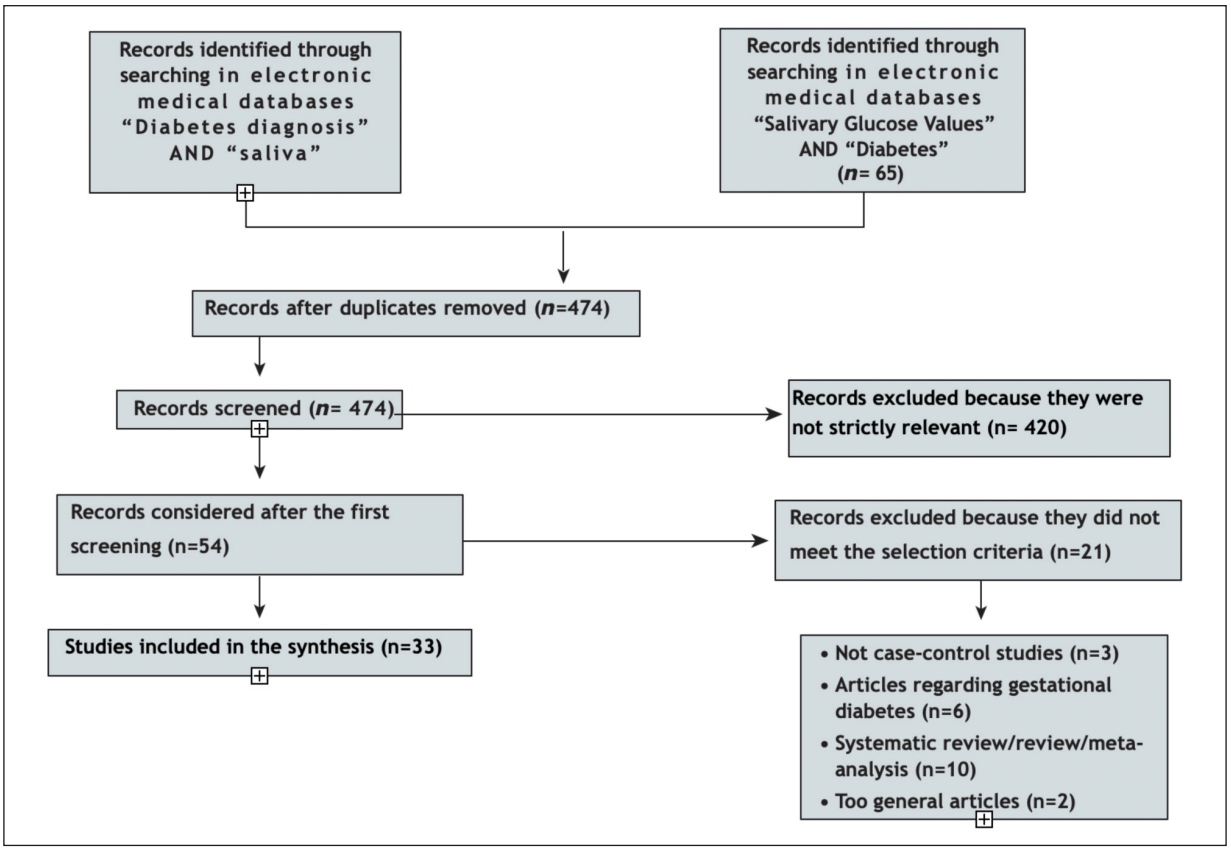

Figure 1. Literature search flowchart (PRISMA).

evaluated 82 patients, then 1 study[36] on resistin and involved 73 patients, 1 study[50] on fructosamine and examined 100 patients and, lastly, 1 considered CPS1[37] and evaluated 69 patients.

From these studies, the reported markers proved to be potentially useful for diabetes screening and diagnosis, with the exception of the Techatanawat et al[35] study, which concluded that salivary cystatin SA levels might not be affected by glycemic status. This study concluded that the salivary level of cystatin SA was associated with the severity of periodontal disease, but not with glycemic status.

Finally, 10 additional articles[38-45,47,49,51] dealt with multiple markers simultaneously, such as salivary glucose, amylase, urea, Mg, Ca, Zn, and phosphorus, and in total, they considered 1,732 patients. In all these studies, it is clear that saliva could be suggested as a useful diagnostic tool for DM because it can reflect blood parameters. Tiongco et al study[22], whose aim was to analyze glucose, amylase, calcium, and phosphorus as possible markers, reported that only salivary glucose and amylase showed good potential in discriminating patients with diabetes from healthy ones. Then, Cui et al[43] concluded that the efficacy of salivary glucose as a diagnostic marker for DM is dependent on standardized salivary preanalysis, collection, and processing methodologies. Standardized saliva collection and processing techniques are key to minimizing interindividual variability in saliva composition.

Discussion

This systematic review aimed to verify if saliva can be considered a useful tool for the early diagnosis of DM. While some authors believe that saliva tests have the potential to become an effective and non-invasive method for the diagnosis or monitoring of DM, others strongly reject such a hypothesis. The review takes into consideration various salivary biomarkers in order to understand whether these may be associated with health or disease status.

Most of the literature that investigated salivary glucose report that this biomarker can be useful for distinguishing non-diabetic subjects from diabetic patients. There was heterogeneity in the studies analyzed with regard to the population, the methods of collection of the saliva sample, and the way in which the results were presented, so it is difficult to compare the results of the various studies.

For example, Samar Fares et al[17] considered a population aged between 18 to 65. They did not describe the method by which the saliva was collected, and the results indicated that salivary glucose was significantly correlated to FBS with strong positive association ($r=0.67$, $p<0.001$ in control group, $r=0.56$, p-value<0.001 in diabetic group and $r=0.36$, $p = 0.01$ in pre-diabetic group). Salivary glucose could differentiate non-diabetics from diabetics (AUC: 0.928, $p<0.001$) with sensitivity (94.2%) and specificity (62%) and differentiate non-diabetics from pre-diabetics (AUC: 0.928, $p<0.001$) with sensitivity (94.2%) and specificity (62%)[17].

The study by Satish et al[18], on the other hand, included patients in the 25-45 age group. Blood and salivary samples were collected during resting conditions. The unstimulated saliva was collected from both the diabetic and control groups by the method of spitting, while the subject was fasting. All the individuals were asked to wash their mouths thoroughly before the collection of salivary samples.

Fasting blood samples were also collected from both the diabetic and control groups by venipuncture technique. In the study of Kumar et al[23] the results showed a significant correlation ($r = 0.54$) and ($r = 0.45$) between fasting blood glucose and fasting salivary glucose for the diabetic group and control group, respectively[18].

Patients over 31 years of age were selected in the study by Tiongco et al[22]. In this article, unlike the other two mentioned above, the inclusion and exclusion criteria are well-described. Prior to specimen collection, participants were asked to fast for a period of 6 to 8 hours, and before collection of saliva, participants rinsed their mouths twice with distilled water. Participants were instructed to spit the pooled saliva in a sterile, disposable plastic container over a period of 5 minutes, then samples were stored on ice and sent to the laboratory immediately. Fasting blood samples were also taken from all participants under aseptic conditions. In this study, a significant correlation between blood and salivary glucose was observed ($r = 0.715$, $p < 0.001$). Further analysis also showed that salivary glucose is 88.5% sensitive and 61.5% specific, with a positive predictive value of 45.8%, and a negative predictive value of 97.1%[22].

The study by Gupta et al[19] considered a total number of 47 Type 1 diabetics between 10-55 years, and 165 Type 2 diabetics between 35-80 years. The control group consisted of 38 individuals, with

a negative history of diabetes mellitus between 15-47 years. Samples were collected in the morning between 9 and 11 a.m. from patients who were in a fasting state. First, a drop of blood was collected for fasting glucose estimation by pricking the middle finger, then a drop of blood oozing from it was absorbed by a disposable blood glucose test strip. The strip was then placed into the glucometer and the reading of blood glucose was recorded. Regarding the collection of saliva, the patients were made to sit upright in a comfortable position and were asked to rinse the oral cavity using plain water. The saliva secreted for the initial 2 minutes was swallowed and, subsequently, secreted saliva was held in the oral cavity for the following 5 minutes. Then, the saliva pooled in the floor of the oral cavity was collected through the suction technique (using a syringe). Finally, this collected salivary sample was preserved in a box with ice and then transported to the laboratory within two hours.

The Pearson correlation coefficient was 0.073, with a *p*-value of 0.247, not statistically significant[27].

On the basis of the studies considered, salivary glucose could be considered and used as a biomarker for monitoring DM, but before it would be appropriate to carry out studies in which a standardized protocol for collection and storage of blood and saliva samples is established. Furthermore, the results should be presented with more homogeneity in order to compare the different articles and establish the real usefulness of salivary glucose in this field.

In addition to glucose, other biomarkers have been studied. All the studies, with the exception of the one relating to cystatin SA[35], have shown encouraging results; however, to date, there is not enough literature to state that these biomarkers are effectively useful for the diagnosis of DM.

Concerning 1-5 anhydroglucitol, only two studies considered have investigated these biomarkers. Mook-Kanamori et al[31] considered subjects aged between 30 and 70 years, while the controls were between 23 and 63 years. Both groups were of Asian and Arab ethnicity. On the other hand, Chaohui Jian et al study[30] selected subjects older than 18. In both studies, saliva was obtained using the Salivette system following the manufacturer's recommendations. After collection, the samples were stored on ice and then transferred to a -80°C warehouse. Finally, in both studies, a mass spectrometry-based method was used for the quantification of 1,5-AG.

Aprosin, alpha amylase, N-acetyl-β-D- hexosaminidase, resistin, fructosamine e CSP1[32-34,36,37,50] were investigated by only one study each. In general, all articles concluded that these biomarkers can be considered useful for the diagnosis of DM. Therefore, it would be appropriate to expand scientific research to establish whether they can actually be used at a clinical level. For example, in the alpha-amylase study[32] a total of 80 participants in the 30-60 age group was selected. Samples of unstimulated saliva were collected between 9 and 11 a.m. to avoid diurnal variation and stored at 4°C until analysis. Antecubital venous blood samples were taken after 12 hours of overnight fasting from each individual. Subsequently, each sample was centrifuged. The study concludes by confirming the significant increase in salivary amylase levels in diabetic patients compared to healthy individuals. However, it is emphasized that studies with a larger sample size, including prediabetics, type I diabetics, and type II diabetics in all age groups, are needed to validate these findings.

Conclusions

In conclusion, once it has been ascertained that a salivary biomarker is really useful for the diagnosis of DM, it would be necessary to define precise values for each marker in order to predict with reasonable confidence if an individual is healthy or suffering from diabetes.

Finally, as argued in Cui et al[43] study, standardized saliva collection and processing techniques are key to minimizing interindividual variability in saliva composition.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

The authors confirm their contribution to the paper as follows: study conception and protocol design for scoping review: NC, FC, and CM; data collection: NC, FC, and CM; analysis and interpretation of results: NC, FC, CM, MDF, and GMT; draft manuscript preparation: NC, FC, CM, MDF, and GMT. All authors reviewed the results and approved the final version of the manuscript. NC, FC, CM, MDF, and GMT confirm sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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Ethics Approval and Informed Consent
Not applicable.

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