

Review

Saliva as a biomarker for estimation of glucose level in healthy individuals, a systematic review

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Abstract

According to the International Diabetes Federation, if no actions are taken, the number of people with diabetes could grow to 629 million globally by 2045. The most widely used tool for diabetes detection and diagnosis is currently the measurement of serum glucose levels. Saliva can be a reliable non-invasive diagnostic biomarker for determining glycaemic status in individuals. The study's objective is to systematically analyze articles to determine whether saliva can be used as a biomarker to test glycemia as a non-invasive process for correlating salivary glucose and blood glucose in healthy subjects. The study's question was, "Can saliva be used as a potential alternative biomarker to detect glycemia?" An electronic search was conducted in the PubMed, Google scholar & Wiley online library databases using MeSH terms – "salivary biomarker", "salivary glucose", "blood glucose", "healthy individuals", and "adults" from the year 2015 to 2020. The quality of the study was assessed using the QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies-2) tool. A total of 4680 articles were retrieved from the electronic database folder. After a complete evaluation of the title, the abstract and the full text of the articles, 16 were selected for review. Using the QUADAS-2 tool, all the articles were classified as low-risk bias. The findings suggest a positive correlation between the amount of salivary glucose and blood glucose from the data obtained from the studies which have been retrieved. The salivary and blood glucose amount confirms its use in healthy individuals as a non-invasive diagnostic biomarker for finding glycaemic status.

Keywords: salivary biomarker, salivary glucose, blood glucose, healthy individuals and non-invasive method.

Introduction

Rationale

According to the International Diabetes Federation (IDF), if nothing is done, the number of people with diabetes could grow to 629 million globally by 2045. In 2015, diabetes was ranked sixth and seventh among the top 10 leading causes of death worldwide and in SEAR [1]. The most widely used tool for diabetes detection and control is currently the measurement of serum glucose levels by extracting blood from the peripheral veins, which is invasive and painful [2]. Studies have shown that the prevalence of anxiety induced by the fear of being pricked by the needle is 22% in people regardless of

their age and therefore discourages them from testing for the amount of sugar on a daily basis [3]. It has been estimated that 20.5% of the population refuses any medical attention due to needle prick anxiety [4]. There are plenty of illustrations of the justification for using saliva in hyperglycaemia control. Glucose is a compact molecule that, through semi-permeable diaphragms, can easily spread. In diabetic patients, changes in the basement membrane are observed in blood vessels that allow saliva to increase the distribution of blood glucose [5]. Saliva is a biological fluid that is ultra-filtrated of serum capable of representing both changes at the local and systemic level because the secretion composition is affected by hormonal, immunological, medical, psychological, and personal metabolic conditions



[6, 7]. The stimulated salivary flow is 7ml/min and the unstimulated salivary flow rate is 3ml/min [8]. The incidence of caries, periodontal illness and candidiasis will increase with an increase in specific levels of blood glucose [9, 10]. The collection of saliva should be done as per protocol following the time and method of the collection, which is critical. It has the added benefit of being non-invasive, cost-effective and less technically responsive, thus, reducing discomfort, anxiety and fear among the general population and encouraging healthy individuals to monitor the level of glucose on a regular basis [11, 12].

Objectives

This study aims to systematically analyze articles to determine whether saliva can be used as a biomarker to test glycemia as a non-invasive process for the correlation of salivary glucose and blood glucose in healthy subjects.

Null hypothesis: The glucose content in saliva and serum is not substantially related. Alternate hypothesis: The glucose content in saliva and serum is substantially related.

Material and methods

Protocol and registration

The systematic review was done following the guidelines of PRISMA DTA (Preferred Reporting Items of Systematic Review and meta-analysis–diagnostic test accuracy) guidelines [13] were registered in PROSPERO – CRD42020207954. The study question was, “Can saliva be used as a potential alternative diagnostic fluid to detect glycemia?”

Eligibility criteria

The systematic review is written in the English language according to PECOS – Healthy individuals with the age group of >18 years without any past medical

illness or medicine which affects the salivary flow or composition were included (P-Population), salivary glucose level was being assessed (E-Exposure). The comparators included were the blood glucose level (C-Reference test). The outcome of the following study is that salivary and blood glucose levels have been positively correlated with saliva as a non-invasive biomarker for glycemia assessment (O-Outcome). The study design included *ex vivo* studies and observational studies (S-Study design). All languages other than English literature articles were excluded. Animal experiments, textbooks, reviews, and thesis were excluded from the studies.

Information sources

A search was conducted to investigate whether salivary glucose can be used as a non-invasive method to detect glycemia using electronic databases of “PubMed”, “Wiley online library” and “Google scholar”. Most of the relevant *ex vivo* studies that explained the association between salivary glucose levels and blood glucose from 2015 to 2020 have been included in the study (Table 1).

Study selection

The data was reviewed for the following title, abstract, keywords: salivary glucose level, blood glucose level, healthy individuals and adults.

Data collection process

Data was collected based on author, year of publication, country, population, the method used for salivary glucose estimation, sample size, age, sex, type of observational study, type of saliva, the outcome of statistical analysis and the result.

Definition of data extraction

The data was extracted using electronic databases the PubMed, Google scholar & Wiley online library databases using MeSH (Medical Subject Heading) terms

Table 1: The articles obtained after applying the MeSH terms in the data bases.

Si no.	Database	MeSH Terms	Articles obtained
1.	“PubMed” “Wiley online library” “Google Scholar”	"salivary biomarkers", "salivary glucose level", "blood glucose level", "healthy individuals" and "adults" (year 2015–2020).	4680

–“salivary biomarker”, “salivary glucose”, “ blood glucose”, “ healthy individuals” and “adults” from the year 2015 to 2020. A total of 16 articles were selected to complete the quality assessment. Most studies have used the glucose oxidase peroxidase approach to test the salivary glucose level using a semi-automated analyzer.

Risk of bias and applicability

The study using the Quality Assessment and Diagnostic Accuracy Tool-2 included research on the possibility of bias and applicability problems. The risk of bias was analyzed by one reviewer and taken up by another reviewer for further clarification. All the 16 articles retrieved were classified as low-risk bias. The risk of bias was analyzed by one reviewer (MNH) and taken up by two other reviewers (SPA, SA) for further clarification.

Diagnostic accuracy measures

In all the studies included, the results of the test method were compared to those of a reference method.

The studies were interpreted according to the protocol based on the QUADAS-2 tool.

Results

Study selection

A total of 4680 articles were retrieved from the electronic database folder of PubMed, Google Scholar, and Wiley online library. A number of 89 papers were included after evaluation of the title and 35 articles were selected for further review of the abstract and full text. Only 16 of these papers have been chosen for the Systematic review based on inclusive and exclusive criteria. QUADAS 2 for risk bias assessment of these 16 observational studies was used for qualitative assessment (Figure 1).

Study characteristics

Studies had healthy individuals above 18 years of age who were not under any medication which affected the salivary composition or flow.

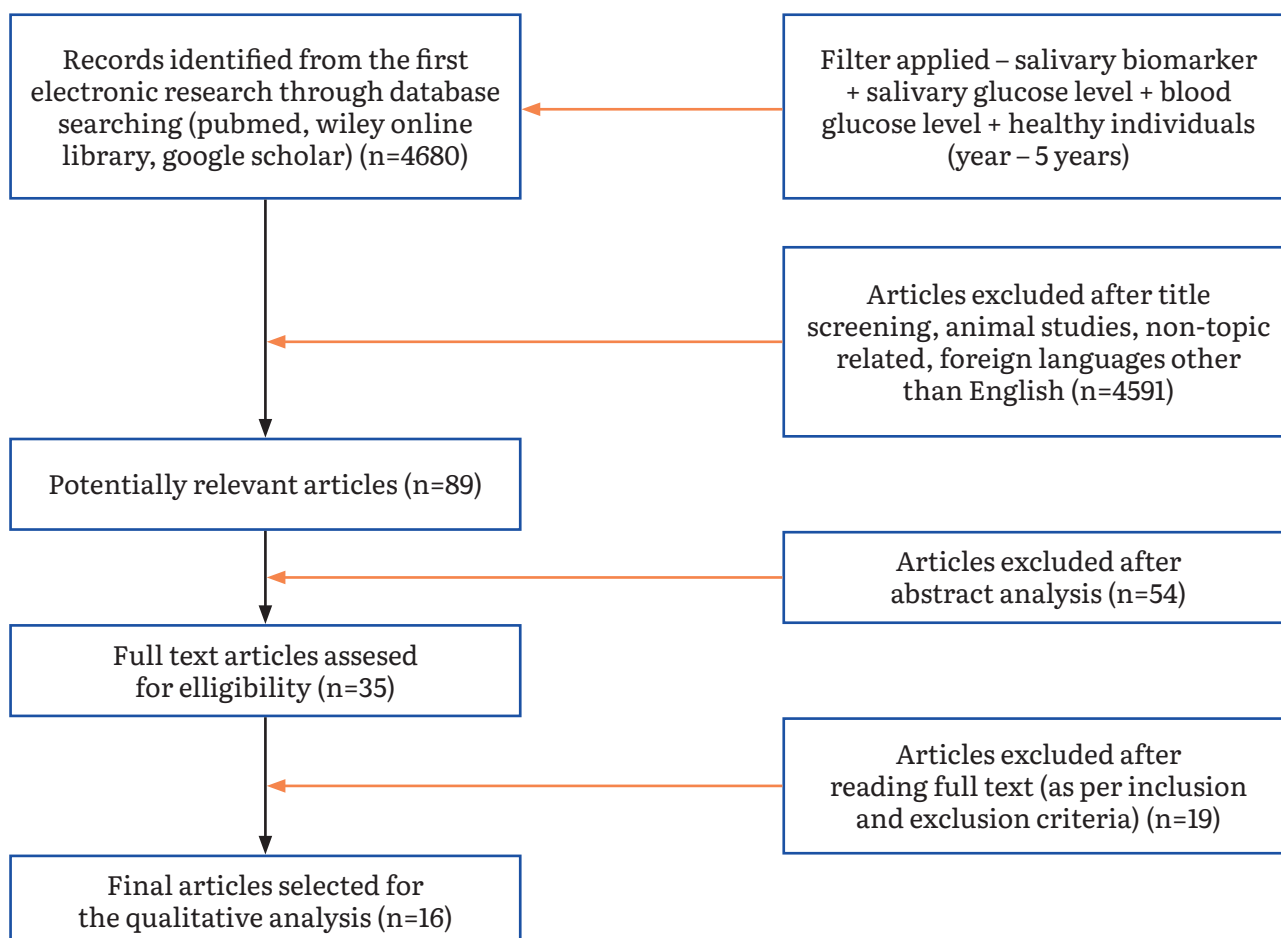


Figure 1: Prisma flowchart – Stages of study selection process.

Risk of bias and applicability

Risk of bias and applicability was done using Quality Assessment of Diagnostic Accuracy Studies (QUADAS 2) tool and the results were tabulated (Table 2).

Results of individual studies

A close correlation has been found between salivary glucose and blood glucose. Studies by Bhalla S et al., 2017 & Bhargava et al., 2018 gave a statistical p-value of 0.0001, while the study by Wang et al., 2017 gives a p-value of <0.01. All the other studies showed a statistical p-value of <0.001 (Table 3).

Discussion

Summary of evidence

A search was conducted to investigate whether salivary glucose can be used as a non-invasive method to detect glycemia using electronic databases of “PubMed”, “Wiley online library” and “Google Scholar”. With the keywords “salivary biomarker”, “salivary glucose”, “blood glucose”, “healthy individuals” and “non-inva-

sive method”. A number of 4680 articles were retrieved from the electronic database folder. Only 16 of these papers have been chosen for the systematic review based on inclusive and exclusive criteria. QUADAS 2 for risk bias assessment of these 16 observational studies was used for qualitative assessment (Figure 1). According to the results of most of the studies, either the spit technique or the passive drool process was used to collect unstimulated saliva. Samples of the saliva were collected 2 hours after breakfast and patients were instructed to sit in the dental chair with their heads turned forward, not to speak, swallow or shift their heads during the operation, and not to swallow any saliva that was present in their mouth. The patient was then advised to spit every minute for 10 minutes in a sterile graduated container and a statistically significant result was shown by the GOD-POD (Glucose Oxidase Peroxidase test) method using the semi-automated analyzer.

In another study, Borg AA, Birkhed D, Berntorp K, Lindgärde F and Matsson L (1998) found that the glucose content in the saliva of the parotid gland increased dramatically in individuals two hours after glucose or food consumption with diabetes mellitus compared to healthy people (Bhalla et al. 2017) [14]. By stabilizing the oral cavity environment, saliva plays a crucial role in maintaining homeostasis of the oral cavity, thereby

Table 2: Risk of Bias and Applicability (QUADAS 2 tool).

Si no.	Domains	Patients selection	Index text	Reference standard	Flow and timing
1	Bhalla S et al., 2017	Low	Low	Low	Unclear
2	N Mishra et al., 2019	Low	Low	Low	Low
3	Kumar S et al., 2020	Low	Low	Low	Low
4	Zhang et al., 2015	Low	Low	Low	Unclear
5	Wang et al., 2017	Low	Low	Low	Low
6	Subha E et al., 2017	Low	Low	Low	Low
7	R Enrique et al., 2017	Low	Low	Low	Low
8	Ladgothra et al., 2016	Low	Low	Low	Low
9	M Hossein et al., 2016	Low	Low	Low	Low
10	Ravindran et al., 2015	Low	Low	Low	Low
11	Vishwanath et al., 2017	Low	Low	Low	Unclear
12	R Enrique et al., 2019	Low	Low	Low	Unclear
13	S Fares et al., 2019	Low	Low	Low	Unclear
14	Arora et al., 2015	Low	Low	Low	Low
15	Bhargava et al., 2018	Low	Low	Low	Unclear
16	S Gupta et al., 2017	Low	Low	Low	Unclear

Table 3: Synthesis of results.

Si no.	Author and year	Country	Population	Method	Sample size	Age & gender	Type of study	Statistics	Type of saliva	Results
1	Bhalla <i>et al.</i> , 2017	India	Peri-urban	GOD-POD	500	8-95 (not mentioned)	Case control	Mann-whitney test	Unstimulated saliva	Saliva may be used as an adjunct diagnostic tool in DM
2	N Mishra <i>et al.</i> , 2019	India	Urban	GOD-POD	200	>40 (not mentioned)	Case control	Z test	Unstimulated saliva	Positive correlation between salivary glucose and blood glucose level
3	Kumar S <i>et al.</i> , 2020	India	Urban	GOD-POD	90	Not mentioned	Case control	Anova	Unstimulated saliva	Saliva can be used to access the diabetic status. Increased salivary glucose level leads to increased oral candidal carriage
4	Zhang <i>et al.</i> , 2015	USA	Urban	Saliva nano-bio-sensor	2	20-30 (not mentioned)	Not mentioned	Not mentioned	Not mentioned	Saliva can be used as a non-invasive method
5	Wang <i>et al.</i> , 2017	China	Urban	GOD-POD	60	47-84 (28M, 32-F)	Case control	Student t test	Not mentioned	Saliva as a non-invasive method for monitoring blood glucose level
6	Subha E <i>et al.</i> , 2017	Dubai	Urban	GOD-POD	40	35-64 (20-M, 20-F)	Case control	Student t test, chi-square test	Unstimulated saliva	Saliva as a non-invasive method for monitoring blood glucose level
7	R Enrique <i>et al.</i> , 2017	Phillipines	Urban	GOD-POD	75	31-61 (25-M, 50-F)	Case control	Anova	Unstimulated saliva	Salivary glucose as a non-invasive biomarker for diagnosing screening and monitoring type 2 diabetes
8	Ladgothra <i>et al.</i> , 2016	India	Peri-urban	GOD-POD	120	35-65 (73-M, 47-F)	Case control	Not mentioned	Unstimulated saliva	Salivary glucose as a non-invasive for monitoring diagnosing diabetes

Note: DM – Diabetes Mellitus; GOD-POD – Glucose Oxidase Peroxidase test.

Table 3: COntinued.

Si no.	Author and year	Country	Population	Method	Sample size	Age & gender	Type of study	Statistics	Type of saliva	Results
9	M Hossein et al., 2016	Iran	Urban	GOD-POD	30	24-59 (17-M, 13-F)	Case control	Anova	Unstimulated saliva	Saliva can be used as an alternative to serum for diagnosis for DM in oral glucose tolerance test
10	Ravindran et al., 2015	India	Peri-urban	GOD-POD	60	Not mentioned	Case control	Student t test	Unstimulated saliva	Salivary glucose and PAS positive cells are increased in diabetes which can be considered as an adjuvant diagnostic tool for DM
11	Vishwanath et al., 2017	Korea	Urban	Glucose bio-sensor	Not mentioned	Not mentioned	Case control	Not mentioned	Unstimulated saliva	Salivary glucose can be used as a non-invasive diagnostic method
12	R Enrique et al., 2019	Phillipines	Urban	GOD-POD	80	>18 (Not mentioned)	Cross sectional	Mann-whitney test	Unstimulated saliva	Salivary glucose and amylase showed good potential in discriminating patients with diabetes from those without diabetes.
13	S Fares et al., 2019	Egypt	Urban	GOD-POD	100	Not mentioned	Case control	Anova	Unstimulated saliva	Salivary glucose correlated with fasting blood sugar with positive association with non-diabetic group and with medium positive association in pre-diabetic group.

Table 3: CContinued.

Si no.	Author and year	Country	Population	Method	Sample size	Age & gender	Type of study	Statistics	Type of saliva	Results
14	Arora <i>et al.</i> , 2015	India	Urban	GOD-POD	200	Not mentioned (M-111, 89-F)	Case control	Z test	Unstimulated saliva	Saliva as a non-invasive and reliable marker for the prediction of glucose level in type 1 diabetes
15	Bhargava <i>et al.</i> , 2018	India	Urban	GOD-POD	40	Not mentioned	Case control	Un-paired t test	Unstimulated saliva	Salivary glucose level can be used as an index of diabetes mellitus
16	S Gupta <i>et al.</i> , 2017	India	Urban	GOD-POD	120	20-75 (Not mentioned)	Case control	Kruskar wallis test	Unstimulated saliva	Salivary glucose level can be used for monitoring glycemic level in diabetes mellitus

acting as one of the markers for the successful treatment of disease and risk estimation (N Mishra et al., 2019) [15]. In people with diabetes that are under surveillance, salivary glucose levels have a very high correlation coefficient ($r=0.841$) and a statistically strong, important correlation ($P<0.001$) with the amount of blood glucose.

After an increase in blood glucose after a glucose load, the research of Kortuem and Shannon et al. recorded an increase in salivary glucose, indicating similar findings as in our research. (Kumar S et al., 2020) [16]. The viscosity of human saliva varies between individuals and is easily affected by the biological environment (Zhang et al., 2015) [17]. Advances in medical technology have immense potential to achieve the long-term objective of scientifically validated health screening based on saliva and early warning tests for oral disease and other systemic conditions (Wang et al., 2017) [18]. Amir S et al. indicate that glucose was only found in the saliva of patients with diabetes mellitus, while glucose was not present in salivary samples of age-matched non-diabetic subjects (Subha E et al., 2017) [19]. Therefore, saliva testing surpasses all the drawbacks of venepuncture and provides all age ranges with ease of testing (R Enrique et al., 2018) [20]. Elevated salivary glucose levels are responsible for diabetic membranopathy, which leads to leakage through the basement membrane and increases glucose percolation from blood to saliva (Ladgothra et al.) [21].

The oral glucose tolerance test for unstimulated whole saliva was investigated and we found that the salivary glucose level increased within 60 minutes during the glucose tolerance test and decreased after 2 hours in healthy participants and was high in diabetic patients, as was observed in serum (M Hossein et al., 2016) [22]. Compared to fasting serum glucose levels in the diabetic population, a corresponding rise in fasting salivary glucose levels was observed, which was statistically important. ($p=0.031$) (Ravindran et al., 2015) [23]. Salivary glucose levels have shown a significant association between study groups with plasma glucose levels, suggesting that saliva glucose levels can be used as a glucose control tool in diabetic patients (Vishwanath et al., 2017) [24]. The glucose molecule is small and can spread easily across the semi-permeable membrane, leading to increased leakage of saliva glucose (R Enrique et al., 2019) [25]. In the Takeda et al. research, salivary chemical concentrations were measured in healthy subjects under varying conditions and almost all metabolites were found to be higher in unstimulated saliva than in stimulated saliva (S Fares et al., 2019) [26]. Ivanovski

et al. conducted a study among xerostomic diabetic patients and concluded that even if the patient had xerostomia, which is a complication of diabetes, the association between blood and salivary glucose levels was statistically important (Arora et al., 2015) [27]. Salivary and blood glucose are significantly associated and will be useful in controlling diabetes. Therefore, given that today's glucose monitoring procedures are invasive, saliva can act as an alternative non-invasive diagnostic fluid to help solve this problem. Therefore, saliva testing exceeds all the drawbacks of venepuncture and makes it possible to test for all age groups (Bhargava et al., 2018) [28]. Saliva plays a major role in oral cavity homeostasis because it stabilizes the oral cavity environment and thus serves as a brilliant marker for the timely discovery of the disease, further contributing to more successful treatment, risk assessment, glucose level assessment and a quick, non-invasive alternative blood and urine test (S Gupta et al., 2017) [29].

The study given by Zhang et al. had a limited sample size within a range of 2, while the remaining studies had an appropriate sample size to confirm the usefulness of saliva as a diagnostic marker. There is no established standard method and time for the collection of saliva and a standard normal range of salivary glucose level as a non-invasive biomarker related to the age of the healthy population.

Conclusion

The findings suggest a positive correlation between the amount of salivary and blood glucose from the data obtained from the studies which have been retrieved. The salivary and blood glucose amount confirms its use in healthy individuals as a non-invasive diagnostic biomarker for finding glycaemic status.

Conflict of interests

The authors declare no conflict of interest.

Ethical approval

This article contains no studies with human participants or animals performed by any of the authors. All applicable international, national and/or institutional guidelines for care and use of animals were followed. All procedures performed in studies involving human

participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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