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Original Article

Estimation of salivary glucose, amylase, calcium, and phosphorus among non-diabetics and diabetics: Potential identification of non-invasive diagnostic markers

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ABSTRACT

Aims: Diabetes Mellitus (DM) continues to burden millions of people worldwide. Early detection and effective diagnosis of DM are essential key strategies to reduce the impeding incidence of the disease and its complications. Thus, this study determined the potential utility of salivary glucose, amylase, calcium, and phosphorus as non-invasive diagnostic markers of DM.

Materials and methods: A total of 80 participants were recruited and divided into two groups (non-diabetics and diabetics). Fasting blood samples and unstimulated saliva samples were collected and tested for glucose, amylase, calcium, and phosphorus.

Results: Mann-Whitney *U* test shows that salivary glucose and salivary amylase were significantly higher among diabetics than non-diabetics. In addition to this, the receiver operations characteristics (ROC) curve showed that salivary glucose (AUC = 0.811, $p < 0.001$) and amylase (AUC = 0.649, $p = 0.03$) has significant association with DM.

Conclusion: Overall, only salivary glucose and amylase showed good potential in discriminating patients with diabetes from those without.

1. Introduction

Diabetes mellitus (DM), a silent epidemic, is drastically increasing in prevalence and continues to burden millions of people worldwide. According to the International Diabetes Federation (IDF) in 2017, approximately 425 million individuals are affected by this silent killer. Moreover, these numbers are expected to rise by about 48% by the year 2045. Of the different IDF regions identified, the Western Pacific region has the highest number of recorded cases with 159 million individuals afflicted. This number is still expected to rise by 15% in the year 2045. The Western Pacific region also recorded the highest number of deaths due to diabetes, with an estimated 1.3 million deaths per year [1,2].

Currently, diabetes mellitus is diagnosed by estimation of fasting blood sugar, 2-h post-prandial blood sugar, random blood sugar, or hemoglobin A_{1c} [3]. It is crucial to identify high-risk individuals at an earlier stage in order to reverse any overt changes and control the growing epidemic [4]. Population-based screening for DM could be useful in the prevention and early treatment of patients. Although several diagnostic tools are available for early identification of DM risk, new diagnostic tools are still needed to complement current diagnostic measures for predicting DM incidence. Well-established measures of DM, such as, fasting blood sugar and glucose tolerance tests, require blood collection through venipuncture. Even if it is a routine procedure, venipuncture still has some few complications, namely: hematoma, petechiae, thrombosis, vasculitis, infections, and even psychological trauma, such as anxiety and phobia [5].

Recent studies show that the use of saliva as an alternative diagnostic tool to blood offers certain advantages [6]. Since saliva collection is non-invasive and relatively stress-free, saliva can serve as a potential alternative diagnostic sample among infants, toddlers, youth, adults, and the elderly [7]. Human saliva is a unique and dynamic body fluid which is hypotonic and has a slightly alkaline pH of 7.2–7.4. Whole saliva is a complex mixture of proteins, hormones, enzymes, antibodies, antimicrobial constituents,

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and cytokines. The diagnostic potential of saliva is of interest to many researchers due to the presence of multiple disease biomarkers and its non-invasive nature. Although the concentration of these compounds is lowered compared to blood, they can still reflect the body's physiological function [8–11]. Hence, additional studies are required to standardize saliva collection and storage procedures, validate analytical techniques for biomarker detection, and establish reference ranges for routine clinical use [12].

Early and effective screening and diagnosis of DM is an essential key strategy to reduce the impeding incidence of the disease and its complications. Blood testing remains the gold standard in the diagnosis of diabetes. However, due to its invasive nature, it can be painful for some patients and could lead to anxiety and even pose other risks such as infections. Studies regarding the utility of saliva as a diagnostic fluid are promising due to its simplicity, non-invasive nature, and its possible correlation with blood. This study determined the potential utility of salivary glucose, amylase, calcium, and phosphorus as non-invasive diagnostic markers of DM and their correlation with fasting blood glucose (FBG).

2. Materials and methods

2.1. Research design, locale, and respondents

A cross-sectional study was employed to determine the association of the levels of glucose, amylase, calcium, and phosphorus in saliva and serum among selected residents of Angeles City who are non-diabetic and diabetic. A total of 80 respondents were included in this study. Participants are diabetic and non-diabetic males and non-pregnant females living in Angeles City, Philippines aged 18 years old and above. Excluded are those with renal disease undergoing hemodialysis, diagnosed with endocrine disorders, cancer, cardiovascular disease, communicable disease, those undergoing insulin therapy, and pregnant women [13,14]. The respondents were grouped into non-diabetic (ND) and diabetic (D) based on their fasting blood glucose (FBG) levels and the self-reported diagnosis of their physician.

2.2. Blood and saliva collection and processing

Consent of participants was asked before conducting specimen collection. The study and the content of the informed consent were thoroughly explained to each participant. After obtaining their consent and explaining the study to them, they were asked to fast before blood and saliva collection.

Multiple collection tubes were used to obtain blood from the patients. A total of 5 mL of blood was drawn from each participant and placed in a serum separator (gold) tube. After the blood in the gold-top tube has clotted, the gold tube was centrifuged at 3500 RPM for 5 min to extract the serum. On the other hand, unstimulated saliva (i.e., without the use of gustatory, masticatory, or mechanical stimulation) was collected by letting the fluid flow directly into a clean, sterile container. The use of stimulated saliva affects the quantity, pH level of saliva, and the concentration of its constituents [15–17]. About 2 mL of saliva was collected in clean test tubes, which were then centrifuged at 3400 RPM for 10 min, and the supernatant obtained was processed. The specimens collected were immediately tested to maintain the stability and viability of the analyte and to avoid bacterial growth, which can compromise assay validity.

2.3. Biochemical analysis

For serum samples, the conventional enzymatic method was used for measuring glucose, a kinetic assay for amylase and colorimetric assays for calcium and phosphorus determination whereas for the saliva sample, all analytes were assayed using the same reagents utilized for blood samples. As for salivary amylase testing, samples were diluted based on the protocol of Malathi et al. [18].

2.4. Ethical consideration

This study was approved by the Angeles University Foundation-Center for Research and Development Ethics Review Committee (ERC Ref. No. 128) under the title: "***Diagnostic Efficiency of Selected Salivary Analytes as Early and Non-Invasive Biomarkers of Type 2 Diabetes Mellitus***." All participants were informed about the objectives of the study and reassured of the confidentiality and anonymity of data upon signing the informed consent, which served as an agreement between the researchers and the respondents.

2.5. Data analysis

A combination of Microsoft Office Excel and SPSS ver. 23 was used to analyze the data acquired. Mann-Whitney *U* test was used to compare the FBG level and salivary analytes between the study groups. On the other hand, Pearson's correlation was used to determine the association of the various analytes with FBG. Lastly, the receiver operations characteristics (ROC) curve were also drafted to determine the diagnostic potential of the different salivary analytes. All *p*-values are two-sided with a constant 95% alpha-level of confidence and a 5% margin of error.

3. Results

A total of 80 participants were recruited for this study and were divided into non-diabetics (n = 55) and diabetics (n = 25). Summary of their biochemical profile is summarized in Table 1. Mann-Whitney *U* test was used to determine the significant difference between the groups. Among the parameters tested, salivary glucose, and salivary amylase were significantly higher among diabetics than non-diabetics.

Association of the various salivary analytes with diabetes-related parameters was also determined using Pearson's correlation (Figs. 1 and 2). Overall, only salivary glucose ($r = 0.416$, $p < 0.001$) and salivary amylase ($r = 0.226$, $p = 0.04$) showed positive correlation with FBG. This means that when FBG is elevated, levels of both salivary glucose and amylase increase as well.

The diagnostic potential of salivary glucose, amylase, calcium, and phosphorus was determined using the ROC curve, and the results are summarized in Table 2. The ROC curve drafted yielded area under the curve (AUC) values that would indicate the diagnostic potential of the analyte. Based on the results, only salivary

Table 1
Mann-Whitney *U* test analysis of the participants' physical and biochemical profile between non-diabetic and diabetics.

PAREMETER	ND (n = 55)	D (n = 25)	*p*-value
Serum Biochemical Profile			
FBG (mg/dL)	94.1 ± 17.4	174.5 ± 92.7	<0.001*
Amylase (U/L)	92.2 ± 97.2	71.7 ± 21.7	0.87
Calcium (mg/dL)	9.4 ± 9.0	6.8 ± 4.1	0.39
Phosphorus (mg/dL)	2.0 ± 0.7	1.7 ± 0.6	0.07
Salivary Biochemical Profile			
Glucose (mg/dL)	5.7 ± 8.7	12.6 ± 10.5	<0.001*
Amylase (U/L)	613.5 ± 667.3	930.8 ± 827.0	0.03*
Calcium (mg/dL)	3.4 ± 2.7	3.8 ± 2.1	0.14
Phosphorus (mg/dL)	6.6 ± 5.1	7.5 ± 4.7	0.23

FBG: fasting blood glucose; ND: non-diabetic; D: diabetic.
* significant at *p*-value < 0.05.

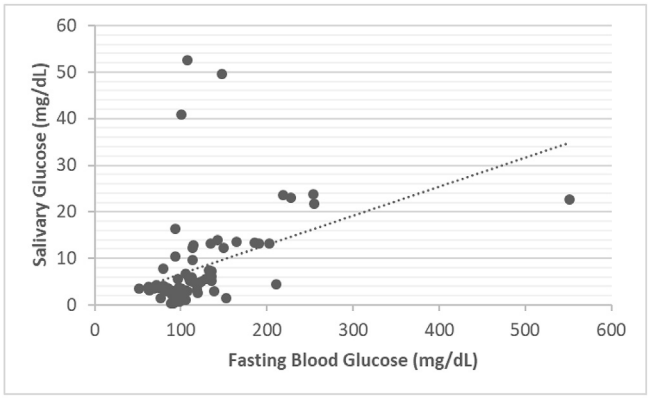

Fig. 1. Pearson's correlation of FBG with salivary glucose levels among non-diabetics and diabetics.

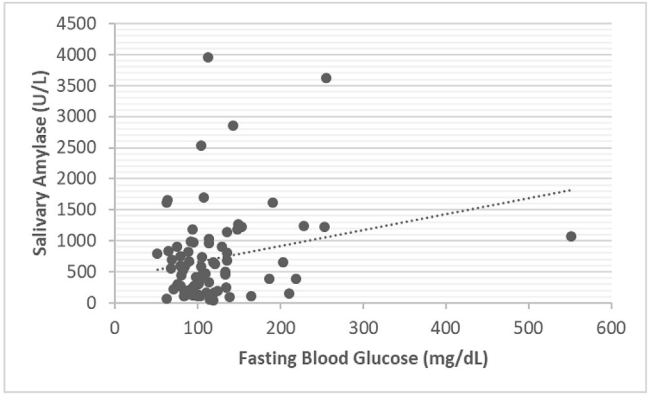

Fig. 2. Pearson's correlation of FBG with salivary amylase levels among non-diabetics and diabetics.

Table 2
ROC curve analysis of the diagnostic potential of salivary glucose, amylase, calcium, and phosphorus in discriminating patients with diabetes from those without.

PARAMETER	AUC	*p*-value
Salivary Glucose	0.811	<0.001*
Salivary Amylase	0.649	0.03*
Salivary Calcium	0.603	0.14
Salivary Phosphorus	0.584	0.23

AUC: area under the curve.
* significant at *p*-value < 0.05.

glucose (AUC = 0.811, $p < 0.001$) and amylase (AUC = 0.649, $p = 0.03$) showed significant association and good potential in discriminating patients with diabetes from those without.

4. Discussion

4.1. Salivary glucose

Various cohorts have already investigated the use of saliva as a substitute for blood. Satish et al. suggest that saliva can be used as a diagnostic fluid for DM for the assessment of glucose, with results showing that there is a positive correlation between salivary glucose and FBG(19). Similar findings showing a strong positive correlation between these two analytes were also observed in many studies where the researchers mentioned that salivary glucose is a good indicator of blood glucose [20–25]. Furthermore, several studies also highlighted the diagnostic and monitoring use of saliva among patients with T2DM [17,19,24,26–29]. Studies have reported that the level of salivary glucose increases in diabetics in comparison to non-diabetic individuals. The excretion of glucose in the saliva is higher among diabetic patients suggesting its use as a potential biomarker for the diagnosis and monitoring of the disease. However, further studies and investigations are needed to fully substantiate and authenticate the use of salivary glucose as an alternative to blood since the studies are subjected to several limitations [22,30]. The proposed mechanism of why salivary glucose concentrations are higher in cases of DM is heavily reliant on the increased leakage of serum-derived components in saliva through the gingival crevices. Diabetic patients have increased basement membrane permeability, which results from microvascular changes in blood vessels due to continuous hyperglycemia. The molecule of glucose is small and thus can quickly diffuse through the semi-permeable membrane, causing the increased leakage of glucose in saliva. Findings of previous studies suggest that this event follows a threshold mechanism most likely involving the parotid gland [19,26,31].

4.2. Salivary amylase

Amylase is the enzyme involved in the hydrolysis of starch into dextrin and monosaccharides composing of glucose units, which may cause hyperglycemia and development of diabetes mellitus [32]. Diabetic patients have altered expression of the amylase and cyclic adenosine monophosphate (cAMP) receptor in their parotid glands, which may lead to changes in secretory protein production of human salivary glands. This may contribute to the altered salivary amylase level associated with diabetes [33]. There is also an association with increased basement membrane permeability and diabetes mellitus, which can also be one of the possibilities for the increased leakage of proteins from the circulation to the oral secretions via the salivary glands of some patients [34]. Results of the present study are similar to previous studies. In the study of Abd-Elraheem et al. [35], the level of salivary amylase was shown to be directly proportional to the level of FBG. The results are also consistent with the study of Prabal Pal et al. [36] and Lopez et al. [37] wherein salivary amylase is significantly increased in the saliva of diabetic individuals. In diabetic patients, which there is high FBG levels, an alteration to the salivary flow is observed. These alterations may lead to the excess leakage of analytes like amylase into the saliva [38–41]. As mentioned in a study by Malathi et al. [18], the levels of salivary amylase among diabetic individuals are elevated due to the altered expression of the enzyme in the parotid glands, and there is also an increased basement permeability of the salivary glands which causes the α-amylase to leak into the individual's secretion easily. Satish et al. [42] also showed a similar result where they found out that there is a significant increase in the levels of salivary amylase in both patients with type 1 and type 2 DM compared to non-diabetics.

4.3. Salivary calcium and phosphorus

The present study was not able to show a significant increase and association in the salivary calcium and phosphorus among diabetic patients compared with the non-diabetics. Although the findings do not offer further support to previous studies where researchers found that salivary calcium is significantly higher among diabetic patients compared with a healthy group [43–45], this is not surprising considering that there are contradicting literature as to the association of salivary calcium and phosphorus among diabetic patients.

Similar to the present results, other studies have also observed

no significant association of both salivary calcium and phosphorus among healthy and diabetic groups [46,47]. The differences in the results of previous studies, including that of the present study may be due to the limited number of participants tested. Thus, the limitation suggests for a larger scale study to better prove an association in the salivary analytes of diabetic patients.

While a slightly higher salivary calcium and phosphorus was noted among the diabetic respondents of the study, a lower serum calcium and phosphorus level was observed. This is similar to the study of Lodgotra et al. where researchers explained that this was due to the decreased insulin, which leads to the stimulatory action of osteoblasts and impairment in calcium homeostasis [45]. The action of insulin is stimulated by vitamin D either by directly enhancing the expression of insulin receptors, thereby increasing the responsiveness of insulin for glucose transport, or indirectly by regulating extracellular cytoplasmic calcium levels [48]. Insulin secretion is a calcium-dependent process which makes patients with T2DM exhibit low insulin levels, thus, decreasing the serum calcium levels. Furthermore, another study noted that the increase in glucose increases urinary excretion of calcium and phosphorus, which is proportional to the degree of glucosuria [46]. Review of literature shows that the increase in salivary calcium among DM patients is due to the reduction in salivary flow rate, or may be due to the increase of the concentration of specific proteins which makes a special bond with calcium phosphate [46]. The presence of calcium in the saliva in high concentration is a beneficial indicator of a person's oral health. Salivary calcium plays a conducive role in enamel remineralization; hence, its saturated levels in saliva [49].

On the other hand, salivary phosphorus levels, regardless of its insignificant result in the study, are higher in diabetics than the other groups. This may be due to the release of the electrolyte in the saliva from the degraded periodontal proteins in T2DM patients and the increased salivary flow rate [45]. In summary, both calcium and phosphorus in the saliva are essential in maintaining the balance between mineralization and demineralization or dissolution of the enamel in the oral cavity [50] and as potential biomarkers of certain diseases.

5. Conclusion

This study comprehensively evaluated the diagnostic potential of salivary glucose, amylase, calcium, and phosphorus among diabetics. It also investigated the possible correlation of the said analytes with their serum counterpart. Overall, only salivary glucose and amylase showed good potential in discriminating patients with diabetes from those without. The two analytes are shown to have a significant positive correlation with FBG. Further studies are still needed to verify our results.

Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2019.07.037.

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