Original Article

Effectiveness of Salivary Glucose in Diagnosing Gestational Diabetes Mellitus

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Abstract

Context: Frequent monitoring of glucose is important in the management of diabetes. A noninvasive painless technique was used to detect glucose levels with the use of saliva as a diagnostic fluid. **Aims:** The aim of our study was to correlate the blood glucose levels with stimulated and unstimulated salivary samples and also to assess the reliability of using salivary glucose in diagnosing and monitoring the blood glucose levels in gestational diabetic patients. **Settings and Design:** The study was conducted among 100 clinically healthy nondiabetic individuals and 99 individuals suffering from gestational diabetes mellitus (GDM). **Subjects and Methods:** Fasting blood glucose estimation and postprandial salivary glucose estimation were done in stimulated and unstimulated salivary samples using glucose oxidase/peroxidase method. **Statistical Analysis Used:** Data obtained were subjected to normality test, and $P \leq 0.05$ was considered to be statistically significant. The correlation between blood and salivary glucose levels was evaluated using Pearson's correlation test. **Results:** A positive correlation was obtained for stimulated and unstimulated salivary samples in fasting and postprandial conditions. Linear regression analysis and receiver operating characteristic curve were plotted, and the optimal cutoff value for unstimulated and stimulated salivary glucose under fasting conditions was 5.1 mg/dl and 5.4 mg/dl, respectively. The optimal cutoff value for unstimulated and stimulated salivary glucose was 8.8 mg/dl and 9.3 mg/dl, respectively, in postprandial conditions. **Conclusions:** Saliva appears to be a reliable biofluid to assess the blood glucose levels and can definitely be a reliable alternative to blood glucose in GDM patients.

Keywords: *Diagnosis, gestational diabetes, saliva, salivary glucose*

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from an absolute deficiency of insulin secretion and/or reduction in the biological effectiveness of insulin or both.[1] Due to the burden of this disease across the globe and in India, diabetes is identified as one of the four priority noncommunicable diseases targeted for action by the United Nations. Various goals and targets have been put forward to reduce the burden of the disease including halting the rise in diabetes, reducing mortality from this disease, and enhancing easy access for patients to affordable basic technologies and essential medication.[2] According to the etiology, diabetes mellitus is classified as type I, type II, gestational diabetes mellitus (GDM), and other specific types.[3]

GDM is increasing in prevalence in most of the developing countries due to overweight and obesity of women in childbearing age. GDM occurs in about 5% of pregnancies and imposes a risk for both mother and child during delivery. Women with a history of GDM also have an increased risk of developing type 2 diabetes mellitus in the years following their pregnancy and also the children have a higher risk of developing type 2 diabetes mellitus (DM) early in life.[4] Hence, it is very important for an early screening, diagnosing, and treating GDM. Screening for preexisting diabetes in very early weeks of pregnancy is important using fasting glucose.

The various screening tests done in GDM include fasting capillary blood glucose, fasting plasma glucose levels, serum fructosamine, and adiponectin. All are assessed using different diagnostic criteria. Glycated hemoglobin level is not a good screening for a DM test, compared to fasting plasma glucose level and oral glucose challenge test.[5] Pregnant women have a higher physiological turnover of erythrocytes, rendering glycosylated

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hemoglobin (HbA1c) inadequate as a diabetic tool because the blood glucose values can be underestimated. In fact, a reduction of HbA1c is seen in normal pregnancy. Oral glucose tolerance test has been used in screening and monitoring patients with GDM. Blood testing remains to be the gold standard in diagnosis, but this can be invasive and painful for most patients and can cause anxiety and fear. Studies have explored the diagnostic value of salivary glucose which is promising due to its noninvasive and its correlation with blood glucose values.

Saliva is a biological fluid that reflects local and systemic changes because the composition of saliva is influenced by hormonal, neurologic, nutritional, and metabolic state of an individual. Higher salivary glucose levels have been reported in diabetic patients compared with levels of nondiabetics. It not only contains glucose but also consists of water, electrolytes, and a variety of proteins such as enzymes, immunoglobulins, albumin, and other biomarkers showing that saliva is functionally comparable to blood in reflecting the physiological status of the body.[6] Till now, many studies were performed to determine salivary glucose as an alternative to blood type 1 and type 2 DM.

The aims and objectives of the study are as follows:

- Aim
  - To assess the reliability of salivary glucose levels as an alternative to blood glucose levels in evaluating the glycemic status of individuals with gestational diabetes.
- Objectives
  - To correlate the blood and salivary glucose levels in gestational diabetic patients
  - To evaluate the variation of salivary glucose both in stimulated and unstimulated saliva and to obtain optimal cutoff values in fasting and postprandial states
  - To calculate the sensitivity and specificity of salivary glucose in predicting the diagnosis and monitoring the glycemic status of an individual with gestational diabetes.

The null and alternate hypotheses of the study were as follows:

- Null hypothesis
  - Salivary glucose cannot be a diagnostic tool in patients with gestational DM.
- Alternate hypothesis
  - Salivary glucose can be used as a diagnostic tool in patients with GDM.

Subjects and Methods

The study was approved by the institutional ethical committee (No. 003/09/2017/IEC/SU), and the written informed consent was obtained from each patient who participated in the study. A total of 199 participants were included in the study. It consists of 100 clinically healthy nondiabetic individuals (sex and age matched) (control group, Group 2) and 99 confirmed diagnosed cases of GDM (study group, Group 1). The patients in the study group were selected according to the criteria for the diagnosis of GDM by the recent guidelines of the American Diabetes Association.[3] Patients with history of smoking or chewing tobacco or alcohol use, any history of salivary gland diseases, previous salivary gland surgery, radiation therapy, patients with any medication altering the salivary flow rate were excluded from the study. The blood and saliva samples from both control and study group participants were collected. 3 ml of blood was collected under aseptic conditions from the antecubital vein of patients after an overnight fasting of 6-8 hours. The collected blood was centrifuged in a sterilized glass test tube at 3500 rpm for 10 minutes. The serum was stored at −20C until analysis.

For saliva collection, patients were asked to rinse the mouth thoroughly with 150 ml of water and sit erect with head slightly down. Standard spitting method was used to collect 3 ml of unstimulated whole saliva into a sterile container which was centrifuged for 15 min at 3000 rpm after which the supernatant was stored at −20C. For collection of stimulated whole saliva, 0.1–0.2 mmol/L citric acid was applied on either side of the dorsal surface of the tongue and saliva was collected using a sterile cup. The collected samples were also treated similar to unstimulated saliva and then stored at −20C. The samples were sent for salivary glucose estimation without any delay. For post prandial samples taken 2 hours after food, blood and saliva were collected in the same way as fasting samples and were subject to glucose oxidase/peroxidase method. This glucose oxidase peroxidase method is based on the principle that glucose is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red-colored quinoneimine dye complex which is read calorimetrically and value is obtained. The values are directly proportional to the concentration of glucose in the samples.

Statistical analysis

The data obtained were compiled and entered in Microsoft Excel sheet and were subjected to statistical analysis using SPSS software (IBM SPSS Statistics for Windows, version 23.0). Normality test was done using the Shapiro–Wilk numerical test, Kolmogorov–Smirnov test, and Q-Q plot test, and all values were normally distributed. $P \leq 0.05$ was considered to be statistically significant. Descriptive statistics for age and gender distribution among the study and control groups were calculated. Student's unpaired *t*-test was used to compare the age, blood glucose levels in fasting, and postprandial states. The mean stimulated and unstimulated salivary glucose values in the study and control groups were also evaluated. Pearson's

Table 1: Mean distribution and comparison of age among study and control groups

Group	*n*	Minimum	Maximum	Mean±SD	*P*	95% Confidence Interval
1 (Study Group)	99	23	40	29.95±4.011	0.538	-1.162 to 2.221
2 (Control Group)	100	18	45	29.42±7.541		

Table 2: Mean distribution of blood glucose among study and control group

Blood Glucose	Mean±SD		Mean Difference	*P*	95% Confidence Interval
	Group 1	Group 2			
Fasting	109.29±28.072	97.69±13.409	11.599	0.000	5.446-17.752
Post-prandial	144.44±42.733	125.19±14.175	19.253	0.000	10.361-28.146

Table 3: Correlation between blood and salivary glucose

Salivary Glucose	Blood glucose	Correlation coefficient (*r*)	*P*
Stimulated fasting	Fasting	0.290	0.000
Unstimulated fasting	Fasting	0.321	0.000
Stimulated post-prandial	Post -prandial	0.409	0.000
Un stimulated post-prandial	Post -prandial	0.414	0.000

correlation test was used to correlate the stimulated and unstimulated salivary glucose in fasting and postprandial states with blood glucose levels in fasting and postprandial states. Linear regression analysis was done, and equation was also obtained. Receiver operating characteristic (ROC) curve was plotted and area under the curve and cutoff values were obtained for stimulated and unstimulated salivary glucose. The sensitivity and specificity for the test and also positive and negative predictive values with positive and negative likelihood ratios were calculated.

Results

The study consisted of two groups, Groups 1 and 2, consisting of 100 individuals in Group 2 and 99 individuals in Group 1. Hence, a total of 199 participants were involved in the study. The mean age of the control group was 29.42 ± 7.541 and that of the study group was 29.95 ± 4.011, respectively, with $P = 0.538$, which was not statistically significant [Table 1].

The mean fasting blood glucose level in Group 2 was 97.69 ± 13.409 and that of Group 1 was 109.29 ± 28.072. The values were statistically significant, with $P = 0.000$. The mean blood glucose level in postprandial states in Group 2 was 125.19 ± 14.175 and in Group 1 patients was 144.44 ± 42.733. The values were statistically significant, with P = of 0.000 [Table 2].

The mean fasting stimulated salivary glucose values in Group 2 patients were 2.103 ± 0.821 and in Group 1 patients was 6.020 ± 0.461, respectively. The mean unstimulated fasting salivary glucose values in Group 2 and Group 1 patients were 1.299 ± 0.625 and 5.373 ± 0.365, respectively. The values were statistically significant, with $P = 0.000$. In postprandial states, the mean stimulated salivary glucose values were 2.103 ± 0.821 and 9.483 ± 0.518 in Group 2 and Group 1 patients, respectively. The mean unstimulated salivary glucose values in postprandial were 1.299 ± 0.625 and 8.919 ± 0.466 in Group 2 and Group 1 patients, respectively. The values were statistically significant, with P = of 0.000 [Figure 1].

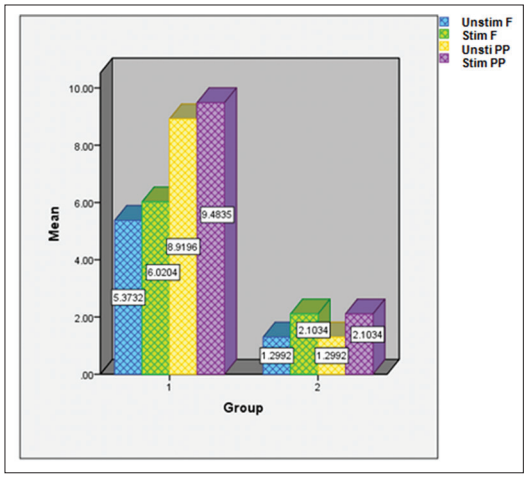

Figure 1: Distribution of salivary glucose among groups

The correlation between blood and salivary glucose levels were evaluated using Pearson's correlation test, and a positive correlation was obtained for stimulated and unstimulated salivary samples in fasting and postprandial conditions. The correlation was poor to moderate, with P value being statistically significant ($P = 0.000$) [Table 3].

The correlation of stimulated fasting salivary and fasting blood glucose samples was evaluated and showed a positive but poor correlation ($r = 0.290$). The linear regression equation with available data was calculated with a model fit $R^2 = 0.084$ and was $y = 90.64 + 3.17$ (stimulated fasting salivary glucose) [Figure 2]. The correlation of unstimulated fasting salivary glucose and fasting blood glucose samples was calculated and showed a positive moderate correlation with $r = 0.321$. The linear regression equation with available data was calculated with a model fit $R^2 = 0.103$ and was $y = 92.03 + 3.44$ (unstimulated fasting salivary glucose) [Figure 3].

The stimulated postprandial salivary glucose levels and postprandial blood glucose levels were correlated

(r = 0.409) and showed a positive moderate correlation. The linear regression equation with available data was calculated with a model fit R^2 = 0.109 and equation derived was y = 1.18E2 + 2.89 (stimulated postprandial salivary glucose) [Figure 4].

The unstimulated postprandial salivary glucose levels and postprandial blood glucose levels were also correlated with r value of 0.414 which showed a positive moderate correlation. The linear regression equation was calculated with a model fit R^2 = 0.111 and regression equation derived was y = 1.2E2 + 2.85 (unstimulated postprandial salivary glucose) [Figure 5].

The ROC curves were plotted and area under the curve with specificity and sensitivity of unstimulated and stimulated salivary glucose levels in fasting and postprandial states was calculated. The fasting stimulated salivary glucose and blood glucose showed 68.4 as area under the curve which was statistically significant with P = 0.000 and a confidence interval of 60.7 and 76.0. The area under the curve implies that the stimulated fasting salivary glucose well distinguishes true positive (diabetes) and true negative (nondiabetes). The sensitivity and specificity for the test were 66% and 63%, respectively, with a positive predictive value of 63.73% and a negative predictive value of 64.95%. The cutoff value for stimulated fasting salivary glucose was 5.4 mg/dl which may translate the idea that patients with value above this are most likely to be diabetic [Figure 6].

The unstimulated fasting salivary glucose and fasting blood glucose were evaluated and ROC curve was plotted with area under the curve 72.0, with a statistically significant P = 0.000. The confidence interval was 64.2 and 79.7. The sensitivity and specificity of the test were 58% and 72%, respectively, with a positive predictive value of 67.06% and a negative predictive value of 63.16%. The cutoff values of fasting salivary glucose were 5.1 mg/dl which extrapolates that individuals having fasting unstimulated

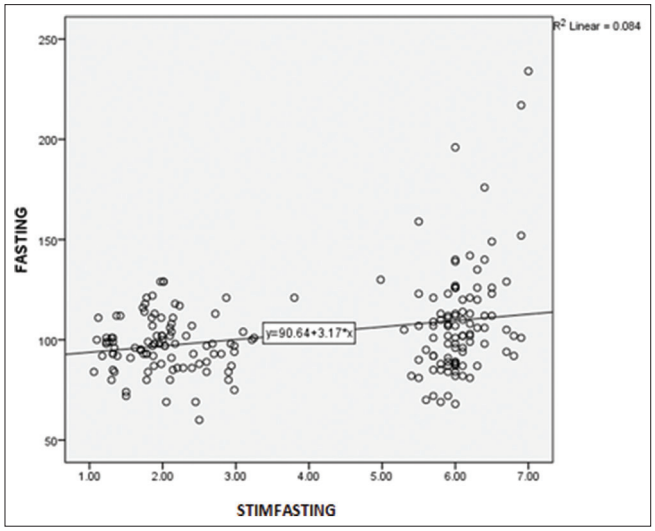

Figure 2: Scatter plot showing correlation and linear regression analysis of stimulated fasting salivary and fasting blood glucose

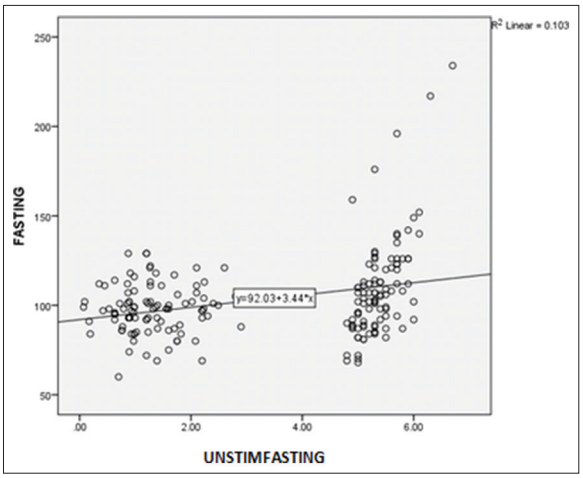

Figure 3: Scatter plot showing correlation and linear regression analysis of unstimulated fasting salivary and fasting blood glucose

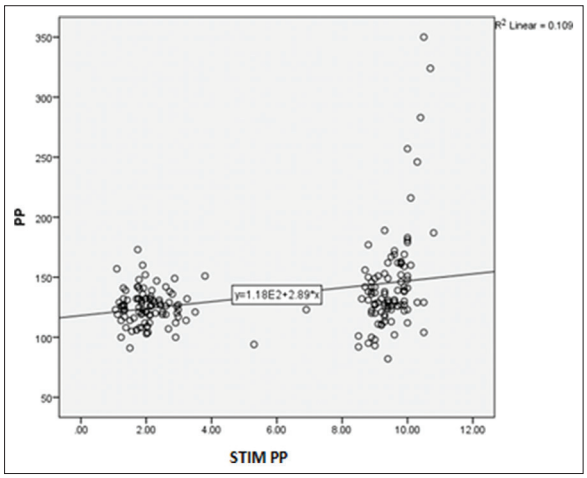

Figure 4: Scatter plot showing correlation and linear regression analysis of stimulated postprandial salivary and postprandial blood glucose

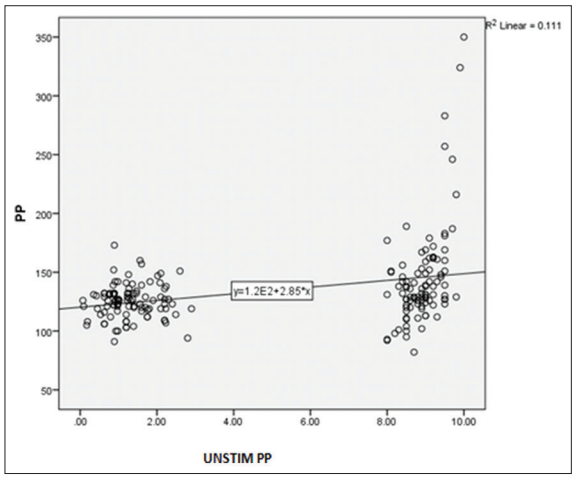

Figure 5: Scatter plot showing correlation and linear regression analysis of unstimulated postprandial salivary and postprandial blood glucose

salivary glucose values above this may have uncontrolled diabetes [Figure 7].

Stimulated postprandial salivary glucose value and postprandial blood glucose values were also evaluated, and ROC curve was plotted the area under the curve as 86.2, with a statistically significant P = of 0.000. The confidence interval was 75.2 and 97.2. The sensitivity and specificity were 82% and 88%, with a positive and negative predictive

Table 4: Sensitivity, specificity and area under the curve for salivary glucose and blood glucose

Blood Glucose	Salivary glucose	Sensitivity	Specificity	AUC	*P*	95% Confidence Interval	Cut-off value
Fasting	Stimulated fasting	66%	63%	0.684	0.000	0.607-0.760	5.4
	Unstimulated fasting	58%	72%	0.720	0.000	0.642-0.797	5.1
Post-prandial	stimulated Post prandial	82%	88%	0.862	0.000	0.752-0.972	9.3
	Unstimulated Post-prandial	82%	87%	0.865	0.000	0.765-0.966	8.8

Table 5: Predictive value and Likelihood Ratio

Salivary glucose	Positive predictive value	Negative predictive value	Positive likelihood ratio	Negative likelihood ratio
Stimulated fasting	0.6373	0.6495	1.7745	0.5451
Unstimulated fasting	0.6706	0.6316	2.0563	0.5892
Stimulated post prandial	0.8710	0.8302	6.8182	0.2066
Un stimulated post-prandial	0.8617	0.8286	6.2937	0.2090

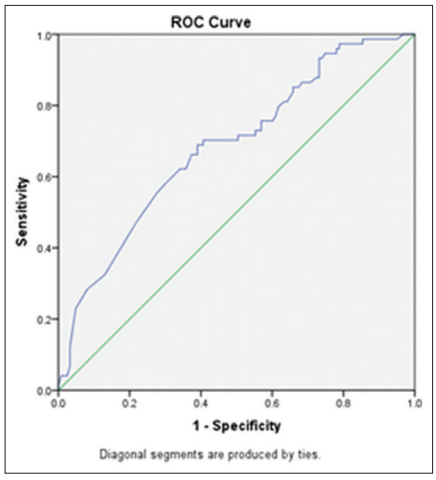

Figure 6: Receiver operating characteristic for stimulated fasting salivary and fasting blood glucose

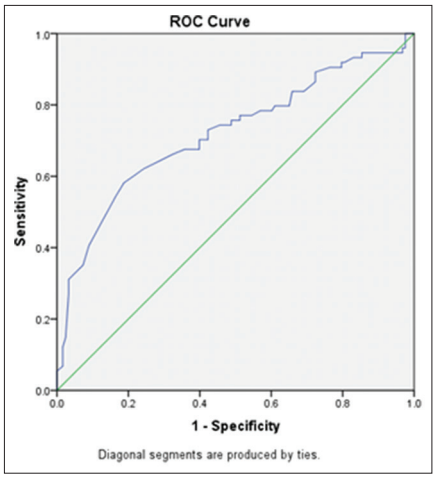

Figure 7: Receiver operating characteristic for unstimulated fasting salivary and fasting blood glucose

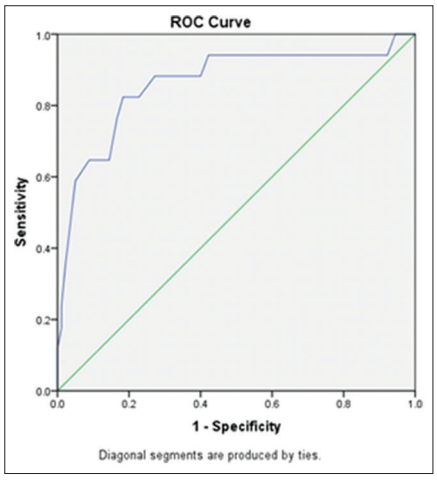

Figure 8: Receiver operating characteristic for stimulated postprandial salivary and postprandial blood glucose

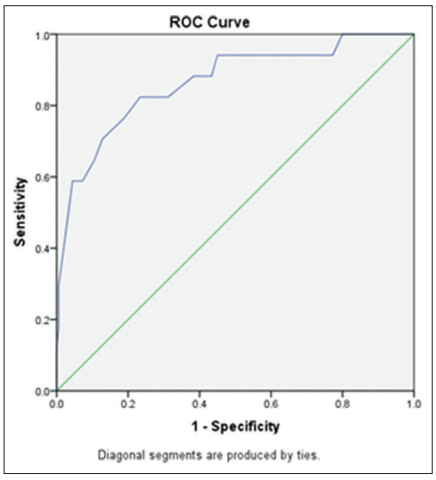

Figure 9: Receiver operating characteristic for unstimulated postprandial salivary and postprandial blood glucose

values being 87.10% and 83.02%, respectively. The cutoff value for stimulated postprandial salivary glucose levels was 9.3 mg/dl above which the patient can be considered diabetic and should go for further investigation to confirm or rule out the disease [Figure 8].

The unstimulated postprandial blood glucose levels and postprandial salivary glucose levels were plotted, and the ROC curve shows the area under the curve as 86.5, with a confidence interval of 76.5–96.6. The *P* value was considered to be statistically significant, with a value of 0.000. The sensitivity and specificity of the test were 82% and 87%, respectively, with a positive predictive value of 86.17% and a negative predictive value of 82.86%. The cutoff value for unstimulated postprandial salivary glucose was 8.8 mg/dl [Figure 9 and Tables 4 and 5].

Discussion

GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.[7] Approximately 7% of all pregnancies are complicated by GDM. This definition also shows the possibility that a woman may have previously undiagnosed diabetes mellitus or may have developed diabetes coincidentally with pregnancy. A woman is diagnosed with gestational diabetes when glucose intolerance continues beyond 24–28 weeks of gestation. The precise mechanisms underlying gestational diabetes remain unknown. The hallmark of GDM is increased insulin resistance. Pregnancy hormones and other factors are thought to interfere with the action of insulin as it binds to the insulin receptor. Since entry of glucose is promoted by insulin, insulin resistance prevents glucose from entering the cell properly. As a result, glucose remains in the bloodstream where glucose level rises.

GDM shows high blood glucose levels similar to all other types of diabetes. Various studies have shown a positive correlation between salivary and blood glucose levels. The important criterion to choose glucose in saliva to measure the blood glucose is that saliva is said to be an ultrafiltrate of blood. Glucose is one of the blood components that are transferable across the salivary gland epithelium in proportion to its concentration in blood. Second, whole saliva is a biological fluid that is simple to collect. The high blood levels of glucose are reflected in the saliva as glucose is a small molecule that can easily diffuse through semi-permeable membranes, thus increasing salivary glucose levels. The advantages of using saliva for diagnosis compared to other biological specimens are the easy availability, simple and noninvasive collection, easy, and painless alternative. Almost any element that can be measured in the blood can be measured in the saliva, thus proving that saliva is an ultrafiltrate of blood and can be used as an alternative to blood in various diseases.[8]

Salivary glucose levels have been correlated with blood glucose levels in various studies performed earlier in both type 1 and type 2 diabetes. There is no study till date in the literature which is available correlating the salivary glucose values with blood glucose levels in GDM. Our study is the first of its kind to evaluate the correlation and also to determine cutoff values for stimulated and unstimulated salivary samples in fasting and postprandial states. The study also aims to check the diagnostic validity of salivary glucose in GDM. As the mechanism of salivary glucose secretion in GDM is the same as other forms of diabetes, the results obtained in GDM patients can be interpreted, and the diagnostic value in GDM can be elucidated.

In the present study, the glucose level in blood and saliva of diabetic patients and healthy controls was measured in fasting and postprandial states. It was found that blood and salivary glucose levels were high in diabetic patients compared to controls, and the difference was statistically significant. The result of our study was in accordance with other studies in type 1 and type 2 diabetes, and the correlation between the blood glucose and stimulated and unstimulated salivary glucose was also showing a moderate positive correlation. The chronic hyperglycemia in DM leads to microvascular structural changes as well as basement membrane alterations in salivary glands. This leads to leaky salivary glands leading to an increase in the glucose diffusion rate from blood to oral cavity. The possible reason could explain the increase in strength of correlation between salivary glucose and blood glucose levels in GDM.[9]

Our study defines the predictive power of salivary glucose to estimate blood glucose levels as well as sensitivity and specificity of the test in GDM. Various other studies done earlier on type 1 and type 2 diabetes showed cutoff values of salivary glucose and predicted the positive and negative predictive values. Since no other earlier studies are available in the literature on salivary glucose and GDM, our results cannot be compared with any other study and directs the path for future research in GDM and salivary glucose.

In our study, the stimulated and unstimulated salivary glucose values in fasting and postprandial states were evaluated with blood glucose values in fasting and postprandial conditions. ROC curves were plotted and area under the curve was evaluated. The area under the curve implies that the unstimulated fasting salivary glucose will distinguish true-positive (diabetes) and true-negative (nondiabetic) patients. The sensitivity and specificity of the test were also evaluated with the positive and negative predictive values along with positive and negative likelihood ratios. The sensitivity of the test varied between 58% and 82% in both stimulated and unstimulated saliva in fasting and postprandial states when calculated separately. The specificity of the test was found to be between 63% and 88% in stimulated and unstimulated saliva under the same condition. There was also a positive predictive value

varying between 63% and 87% and a negative predictive value between 63% and 83%. These values will tell us if salivary glucose would correctly identify the high blood glucose levels. Conversely, false-negative rate was small relative to the true-negative rate. Hence, the probability of an individual having a high blood glucose level with a low salivary glucose level is very low.

Glucose is present in the saliva of normal individuals, however, the mechanism of its secretion is still controversial. Many authors have tried to explain the increased glucose content in salivary secretion of diabetic patients. Salivary glands act as filters of blood glucose and are altered by hormonal or neural regulation.[10] The persistent hyperglycemia can lead to microvascular changes in the blood vessels as well as basement membrane alterations in the salivary glands. This causes increased leakage of glucose from the ductal cells of the salivary glands leading to increased glucose content in saliva.[11] Glucose is a small molecule that easily diffuses through semi-permeable membrane, thereby increasing the salivary glucose levels when blood glucose levels are elevated in diabetes.[12] Complications of diabetes can be due to microvascular changes, and many theories have been put forward to explain the same. Hyperglycemia leads to increased advanced glycosylation end products, commonly known as advanced glycation end products (AGEs). These AGEs cross-link proteins such as collagen and extracellular matrix proteins leading to basement membrane alteration and hence endothelial dysfunction. This alters the microvasculature and makes it more permeable. Furthermore, other products such as the sorbitol diacylglycerol and fructose-6-phosphate formed during hyperglycemia can also lead to basement membrane alteration. The end result, however, is a leaky basement membrane which suggests the increased passage of glucose from blood to saliva in diabetes mellitus.[9] Thus, the presence of glucose in saliva is multifactorial and is not by a single mechanism.

The glucose molecule can easily diffuse via the semi-permeable basement membrane, thereby increasing the glucose levels in salivary secretions and can also be derived from the gingival crevicular fluid into the whole saliva.[13] All these mechanisms can contribute to the presence of increased glucose levels in saliva during elevated blood sugar levels seen in GDM. The results of this study showed a positive correlation between salivary glucose and blood glucose in GDM. The cutoff values of stimulated and unstimulated salivary glucose levels in fasting and postprandial states were evaluated, and this study is one of its kind and the first to derive a regression equation to evaluate the blood glucose levels with a given value of salivary glucose levels in GDM. Further, it also shows how saliva can be used as a reliable substitute for blood in diagnosing patients with GDM.

Conclusion

The outcome of the present study clearly depicts the correlation between salivary glucose and blood glucose levels. Saliva sampling is easy, safe, and noninvasive and can be compared to blood in screening and monitoring GDM. Hence, salivary glucose can be a reliable alternative to blood glucose levels in gestational diabetic patients similar to type 1 and type 2. However, further studies can be performed with a much larger population in different geographic areas to establish the various levels of salivary glucose to diagnose and monitor the patients with GDM.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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