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Salivary and plasma glucose concentrations in type 2 diabetes mellitus compared with healthy volunteers: A case-control study

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Abstract:
Type 2 diabetes mellitus (T2DM) requires regular glucose monitoring, while saliva may provide a convenient and non-invasive alternative to blood testing. This case-control study included 176 participants, comprising 88 patients with T2DM and 88 healthy controls, with fasting and postprandial salivary and blood glucose levels assessed. Salivary glucose levels were significantly higher in patients with T2DM than controls during fasting (3.70 ± 0.59 vs. 0.82 ± 0.15) and postprandially (5.66 ± 1.07 vs. 2.85 ± 0.80; $p<0.001$). Fasting and postprandial salivary glucose correlated positively with corresponding blood glucose levels ($r=0.909$ and $r=0.805$, respectively; $p<0.001$). Thus, salivary glucose may serve as a potential non-invasive adjunct for T2DM assessment, although further studies are needed to establish its diagnostic accuracy.

Keywords: Type 2 diabetes mellitus, salivary glucose, plasma glucose, saliva, non-invasive biomarker, glycemic status, glucose monitoring

Background:
Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, with type 2 diabetes mellitus (T2DM) accounting for most cases and contributing substantially to microvascular and macrovascular complications **[1]**. Accurate assessment of glycemic status is essential for diabetes management, but conventional blood glucose testing requires venipuncture or finger-prick sampling and may cause discomfort **[2].** Saliva offers a non-invasive, easily accessible alternative because salivary glucose has been reported to reflect systemic glucose levels **[3]**. Several studies have demonstrated higher salivary glucose concentrations in patients with T2DM and positive associations between salivary and blood glucose levels, particularly during fasting and postprandial states **[4, 5]**. A systematic review and meta-analysis further reported higher salivary glucose in individuals with T2DM and a positive correlation with blood glucose, although substantial variability was observed across studies **[5]**. Differences in salivary flow, collection methods, patient characteristics, and analytical techniques may contribute to inconsistent findings and limit the clinical application of salivary glucose testing **[6, 7]**. Standardized assessment of fasting and postprandial salivary glucose may therefore help clarify its relationship with corresponding blood glucose levels **[8]**. Further evidence is needed to determine whether salivary glucose can reliably reflect glycemic status and serve as a useful adjunct to conventional testing **[9]**. Therefore, it is of interest to compare the fasting and postprandial salivary glucose with corresponding blood glucose levels in patients with T2DM and healthy individuals and to describe their associations under fasting and postprandial conditions.

Materials and Methods:
A case-control study was conducted at the Department of Oral Pathology and Microbiology, Teerthanker Mahaveer Dental College and Research Centre, Teerthanker Mahaveer University, Moradabad, Uttar Pradesh, India, with participants recruited using a convenience sampling strategy, to determine the difference in fasting and postprandial salivary and plasma glucose levels between the case and control groups. The study involved 176 participants who met the inclusion criteria, of whom 88 were healthy individuals (Group I) and 88 were diagnosed with type 2 diabetes mellitus (Group II), all aged 20–75 years. The sample size was calculated using G*Power software with an effect size of 0.5, an α level of 5% and a power of 95%, which yielded a minimum sample size of 176 (88 per group). The patients with T2DM were further divided into two subgroups according to plasma glucose level: Group IIa comprised 44 patients with plasma glucose between 126 and 200 mg/dl, and Group IIb comprised 44 patients with plasma glucose equal to or higher than 200 mg/dl. The case group included individuals aged 20 to 75 years who met the diagnostic criteria for T2DM, and the control group consisted of individuals of similar age without diabetes mellitus. Participants who suffered from other diseases requiring continuous medication, were pregnant, had mental disorders, were non-cooperative or declined to give informed consent were excluded. Written informed consent was obtained from every participant prior to recruitment, and a medical and demographic history was recorded; the study followed the ethical guidelines and standards of the institution where the research was conducted. Salivary and blood samples were collected under both fasting and postprandial conditions after completion of the clinical examination and after obtaining informed consent. Unstimulated whole saliva was collected in saliva-collection containers, with particular attention paid to proper collection to ensure accuracy and eliminate bias. Venous blood was drawn into vacutainer tubes containing fluoride for the measurement of plasma glucose, again with attention to proper collection and processing to prevent errors. Glucose concentrations in saliva and plasma were estimated using the glucose oxidase–peroxidase (GOD-POD) method, which is based on the oxidation of glucose to gluconic acid and hydrogen peroxide by glucose oxidase, followed by peroxidase-catalysed colour development

that is proportional to the glucose concentration in the sample; the enzymatic reaction was monitored photometrically using commercial GOD/POD assay kits according to the manufacturer's instructions. Fasting and postprandial salivary and plasma glucose levels were determined by this method, and the results formed the basis for statistical comparison between the study groups. The variables studied were fasting salivary glucose, fasting blood glucose, postprandial salivary glucose and postprandial blood glucose. Descriptive statistics (mean and standard deviation) were calculated, and salivary and blood glucose levels in the fasting and postprandial states were compared between the case and control groups, with the level of statistical significance set at $p<0.05$ for between-group comparisons. Pearson correlation coefficients were calculated to assess the strength of the relationship between salivary and blood glucose levels in both the fasting and postprandial states. The final statistical analysis included the data of all 176 participants.

Results:

A total of 176 participants were evaluated, comprising 88 healthy individuals and 88 patients with type 2 diabetes. Among the healthy group there were 55 males and 33 females, and among the patients 51 males and 37 females, so that men slightly outnumbered women in both groups; the mean age was 44.30 ± 13.27 years for men and 44.89 ± 13.12 years for women. In the healthy group, mean fasting salivary glucose was 0.82 ± 0.15 and mean fasting plasma glucose was 82.44 ± 9.78, while mean postprandial salivary glucose was 2.85 ± 0.80 and mean postprandial plasma glucose was 132.66 ± 9.25. In the patients with type 2 diabetes, mean fasting salivary glucose was 3.70 ± 0.59 and mean fasting plasma glucose was 171.15 ± 20.18, while mean postprandial salivary glucose was 5.66 ± 1.07 and mean postprandial plasma glucose was 304.64 ± 42.30 (**Table 1 and Table 2**). Fasting salivary glucose was significantly higher in patients with type 2 diabetes than in healthy individuals (3.70 ± 0.59 vs 0.82 ± 0.15; $p<0.001$), as was postprandial salivary glucose (5.66 ± 1.07 vs 2.85 ± 0.80; $p<0.001$). Similar findings were obtained for plasma glucose: both fasting plasma glucose (171.15 ± 20.18 vs 82.44 ± 9.78; $p<0.001$) and postprandial plasma glucose (304.64 ± 42.30 vs 132.66 ± 9.25; $p<0.001$) were significantly higher in the diabetic group. Using Pearson's test across the whole study population, there was a strong positive correlation between fasting salivary and fasting plasma glucose ($r = 0.909$; $p<0.001$) and a strong positive correlation between postprandial salivary and postprandial plasma glucose ($r = 0.805$; $p<0.001$), so that higher plasma glucose levels were associated with higher salivary glucose levels in both the fasting and postprandial states (**Table 3 and Table 4**). Overall, participants with T2DM showed significantly increased fasting and postprandial salivary and plasma glucose compared with controls, and strong positive correlations were found between salivary and plasma glucose for both conditions.

Table 1: Comparison of mean salivary glucose between the healthy and diabetic groups

Group	Fasting saliva (Mean ± SD)	Postprandial saliva (Mean ± SD)
Healthy	0.82 ± 0.15	2.85 ± 0.80
Diabetic	3.70 ± 0.59	5.66 ± 1.07
P value	<0.001	<0.001

Table 2: Comparison of mean plasma glucose between the healthy and diabetic groups

Group	Fasting plasma (Mean ± SD)	Postprandial plasma (Mean ± SD)
Healthy	82.44 ± 9.78	132.66 ± 9.25
Diabetic	171.15 ± 20.18	304.64 ± 42.30
P value	<0.001	<0.001

Table 3: Correlation between fasting salivary and plasma glucose. Correlation is significant at the 0.01 level (2-tailed)

		Fasting Saliva	Fasting Plasma
Fasting Saliva	Pearson Correlation	1	0.909
	Sig. (2-tailed)		0
	N	176	176
Fasting Plasma	Pearson Correlation	0.909	1
	Sig. (2-tailed)	0	
	N	176	176

Table 4: Correlation between postprandial salivary and plasma glucose. Correlation is significant at the 0.01 level (2-tailed)

		PP Saliva	PP Plasma
PP Saliva	Pearson Correlation	1	0.805
	Sig. (2-tailed)		0
	N	176	176
PP Plasma	Pearson Correlation	0.805	1
	Sig. (2-tailed)	0	
	N	176	176

Discussion:

The present case-control study measured fasting and postprandial salivary and plasma glucose levels in patients with type 2 diabetes mellitus (T2DM) and healthy controls and established an association between salivary and plasma glucose levels. The main findings were that T2DM patients exhibited significantly higher fasting and postprandial salivary glucose levels than healthy controls, and that salivary glucose levels demonstrated strong positive correlations with corresponding plasma glucose levels. The correlation was particularly strong for fasting glucose ($r = 0.909$) and remained notable for postprandial glucose ($r = 0.805$), suggesting that salivary glucose levels can potentially serve as an indicator of blood glucose levels. The present results are consistent with prior research. Panchbhai found that salivary glucose levels correlated positively with fasting blood glucose levels in diabetic patients, and that the correlation was particularly strong in patients with poor glycemic control, suggesting that salivary glucose can be used as an auxiliary tool for assessing blood glucose levels **[10, 11]**. Similarly, studies conducted on patients with T2DM have showed that salivary glucose levels were significantly higher in diabetic patients than in healthy controls **[12, 13]**. The increased salivary glucose levels in diabetic patients can possibly be explained by the increased levels of glucose in the blood, such that a rise in blood glucose is accompanied by a rise in the concentration of glucose in saliva. Moreover, chronically elevated blood glucose levels can damage the salivary gland epithelium and alter its permeability, facilitating the entry of glucose into saliva. However, the mechanisms by which glucose enters saliva are not fully understood and might differ depending on the level of glycemia and the function of the

salivary glands; additionally, variations in salivary flow rate and collection methods can also affect the concentration of glucose in saliva **[14]**. The present study also demonstrated a significant difference in fasting salivary glucose levels between the two groups, with a mean of 0.82 ± 0.15 in the control group and 3.70 ± 0.59 in the diabetic group. This approximately four-fold difference is considerable and suggests that the increase in salivary glucose levels in diabetic patients is not marginal, a result consistent with Mascarenhas *et al.* systematic review and meta-analysis, which showed a large overall effect of T2DM on salivary glucose levels and a positive association between salivary glucose and glycemia **[15]**. The present results are also consistent with prior case-control studies demonstrating that fasting salivary glucose levels can be used to differentiate between diabetic and healthy patients. In one study that included 200 participants, salivary glucose levels were higher in T2DM patients and were positively correlated with serum glucose levels **[16]**. Similarly, salivary glucose levels were found to be higher in both controlled and uncontrolled diabetic groups than in healthy controls and were positively associated with blood glucose levels **[13]**. The strongest correlation was found between fasting salivary and plasma glucose levels ($r = 0.909$; $p<0.001$). This correlation was even stronger than that reported by Mascarenhas *et al.* in their systematic review and meta-analysis, in which the pooled correlation coefficient between salivary glucose and glycemia was $r = 0.49$, rising to $r = 0.67$ in diabetic patients **[15]**. The high correlation coefficient in the present study can be explained by its design, which included a substantial number of participants, was conducted under fasting conditions that reduce interindividual variability, and used a specific protocol. However, correlation coefficients should not be mistaken for diagnostic accuracy, and further research is needed to determine whether salivary glucose levels can be used as a diagnostic tool. Postprandial levels also demonstrated a significant association between salivary and blood glucose levels. Mean postprandial salivary glucose was significantly higher in the diabetic group (5.66 ± 1.07) than in the control group (2.85 ± 0.80; $p<0.001$), and postprandial plasma glucose was likewise much higher in the diabetic group (304.64 ± 42.30) than in the control group (132.66 ± 9.25; $p<0.001$). Moreover, the correlation between postprandial salivary and plasma glucose was strong ($r = 0.805$; $p<0.001$), suggesting that salivary glucose levels can potentially be used to monitor blood glucose after meals; several studies have similarly demonstrated that salivary glucose levels correlate strongly with blood glucose levels in diabetic patients **[11, 13]**. The correlation between postprandial salivary and blood glucose levels was strong but lower than that between fasting salivary and blood glucose levels. This can be explained by the fact that postprandial glucose levels are highly variable and depend on the time since the meal, the caloric content of the meal, gastric emptying, insulin response, peripheral glucose utilization, salivary flow rate and other factors, so that the relationship between salivary and blood glucose levels might not be linear throughout the postprandial period. The present results should also be interpreted in the context of the effects of diabetes on saliva. Diabetes can cause several alterations in the oral cavity, including changes in salivary flow, pH and other parameters. According to the systematic review and meta-analysis by Marques *et al.*, diabetes is associated with significantly lower unstimulated and stimulated salivary flow rates and lower pH, although the quality of evidence was low **[17]**. It is therefore possible that salivary glucose levels are affected by multiple factors in addition to blood glucose levels. The present results should also be interpreted in the context of the methods used to measure salivary glucose. Several studies have demonstrated that salivary glucose levels can vary depending on the collection method, the time of collection, sample storage conditions, laboratory procedures and individual factors. Cui *et al.* found that several donor-related and pre-analytical factors can affect salivary glucose levels and that standardized operating procedures should be established if saliva is to be used for large-scale screening **[18]**, a conclusion supported by another study showing that different saliva collection methods can affect the results of glucose measurements **[19]**. The strong correlations showed by the present study suggest that salivary glucose levels can be used as an auxiliary tool for diagnosing diabetes and monitoring blood glucose. However, the present findings, like prior research, indicate that salivary glucose levels can be used for screening purposes but cannot replace standard blood glucose tests. Several studies have yielded inconsistent results; for example, while Panchbhai found that salivary glucose levels correlated significantly with blood glucose levels, another study of 250 participants found the correlation to be weak and not statistically significant, indicating that further research is needed to clarify the diagnostic utility of salivary glucose levels **[11]**. The clinical utility of measuring salivary glucose is considerable, as the method is non-invasive, relatively painless, inexpensive and potentially suitable for frequent screening **[18, 19]**. It could therefore be used in a variety of settings, including screening in the general population, dental settings, pediatrics and patients who are afraid of needles. However, it is crucial to develop reference intervals, standardized collection and processing procedures and well-established diagnostic criteria before salivary glucose levels can be used for large-scale screening **[20]**. These findings might be particularly useful in a dental setting, as dental professionals often encounter patients who are unaware that they have diabetes or who have poorly controlled diabetes, and a simple salivary test could potentially be used to screen asymptomatic patients. Nevertheless, at present salivary glucose levels can only be used as an auxiliary tool for diagnosing diabetes. A strength of the present study lies in its inclusion of both fasting and postprandial glucose levels, which is a significant advantage over studies that assess only one state, while the relatively large sample size further contributes to the credibility of the findings. The significant differences between the groups and the strong positive correlations found across all comparisons are also notable strengths. The present study has several limitations. First, its case-control, cross-sectional design limits the conclusions that can be drawn, as it is not possible to establish a causal relationship between salivary glucose levels and T2DM or to determine whether elevated salivary glucose

predicts future diabetes. Second, although the study included a substantial number of participants, they were recruited from a single center, which may affect the generalizability of the results. Third, salivary glucose levels can be affected by several variables, including salivary flow, oral hygiene, fluid intake, recent meals and medications, and not all of these were controlled for. Fourth, the study assessed only glucose levels and did not compare them with other established biomarkers of diabetes such as glycated hemoglobin. Fifth, the study demonstrated only an association between salivary and plasma glucose, and a significant correlation does not necessarily mean that salivary glucose can be used interchangeably with plasma glucose; future studies should therefore include receiver operating characteristic analysis, sensitivity, specificity, predictive values and other measures of diagnostic accuracy. Finally, the clinical utility of measuring salivary glucose cannot be fully determined owing to the aforementioned limitations, and comparison across studies is limited by the use of different collection and processing methods and different units of measurement **[18, 19]**. Future research should therefore focus on standardizing the methodology and assessing the reproducibility of the results.

Conclusion:

We show that salivary glucose levels were significantly higher in patients with T2DM than in healthy controls, both in fasting and postprandial conditions and that salivary glucose levels were strongly correlated with plasma glucose levels, particularly in fasting conditions. Thus, data shows that salivary glucose can be used as an auxiliary tool for diagnosing diabetes and monitoring blood glucose levels. However, further research is needed to establish standardized procedures and to assess the diagnostic accuracy of salivary glucose measurements.

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