Salivary alterations in type 1 diabetes mellitus patients: Salivary glucose could be noninvasive tool for monitoring diabetes mellitus

Syed Shahbaz, Girish Katti, Sreenivas Rao Ghali, Chandrika Katti, Darshan D Diwakar, Vijay Guduba

ABSTRACT

Background: Diabetes mellitus (DM) is an endocrine disease characterized by hyperglycemia, the pathogenic mechanisms by which hyperglycemia arises differ widely. Monitoring people with diabetes involve repeated estimations of plasma glucose either by finger pricks or by intravenous blood sampling. Hence, a noninvasive procedure for glucose measurements would be most precious under the circumstances.

Aims: (1) To evaluate salivary glucose, total protein and albumin in type 1 DM (T1DM) patients and to compare with healthy nondiabetic control group. (2) To compare and correlate serum and salivary glucose levels in patients with T1DM.

Study Design: This study consisted of 30 T1DM patients and 30 controls. All subjects were subjected to the serum glucose, salivary glucose, and total protein and albumin estimations.

Materials and Methods: Glucose estimations were done by glucose oxidase-peroxidase method, total protein estimations were done by Biuret method, end point and albumin estimations were done by bromocresol green dye method, end point. All the estimations were performed using an autoanalyzer.

Statistical Analysis: Mean and standard deviation, Student’s t-test and Karl Pearson correlation co-efficient were calculated. All these statistical analyses were performed by using SPSS 11.5 software.

Results: The results showed elevated levels of salivary glucose, total protein and albumin in T1DM group compared to healthy controls. Further the levels of serum and salivary glucose in T1DM patients were significantly correlated.

Conclusion: There are definite changes in salivary composition with increased levels of salivary glucose, total protein and albumin in T1DM patients compared with healthy controls. Salivary glucose could be used for monitoring of DM.

Key words: Albumin, diabetes mellitus, glucose, saliva, total protein, type 1 diabetes mellitus

Diabetes mellitus (DM) is an endocrine disease characterized by a shortfall in the production of insulin with consequent alteration of the process of assimilation, metabolism and balance of blood glucose concentration. Incidentally an array of oral manifestations has been reported in the diabetic population, such as dental caries, unexplained odontalgia, periodontal manifestations, oral mucosal lesions, infections, burning mouth syndrome, and taste disorders. Oral physicians tend to play a pivotal role in detecting and diagnosing this endocrine disease on the basis of the oral signs and symptoms.

Although all forms of DM are characterized by hyperglycemia, the pathogenic mechanisms by which hyperglycemia arises differ widely. It is thus classified, as type 1 DM (T1DM) and type 2 (T2DM). T1DM is a multifactorial disorder that arises following the autoimmune destruction of insulin-producing pancreatic B-cells, leading to severe hyperglycemia necessitating the need for exogenous insulin replacement on a lifelong basis. It was estimated in 2013, that 79,100 developed T1DM annually worldwide. The incidence of T1DM in India is 3/100,000 population.
Salivary alterations in T1DM patients

Monitoring people with diabetes involve repeated estimations of plasma glucose either by finger pricks or by intravenous blood sampling. Hence, a noninvasive procedure for glucose measurements would be most precious under the circumstances. Saliva has long been viewed as a unique yet complex body fluids, like plasma or serum. Saliva is easy to collect by noninvasive methods and preservation is inexpensive. The diagnostic value of saliva lies in its components, flow and structure of glands.

Glucose is a small molecule that diffuses easily through the membrane of the blood vessels, passing through the serum to the gingival fluid by way of the gingival sulcus, and making its way to the saliva. Salivary total protein is a vital component of saliva, predominantly comprising proline-rich proteins. Albumin is regarded as serum ultrafilitrate to the mouth, and it may diffuse into the mucosal secretions.

Noticeably, there is spurge of interest in noninvasive diagnostic testing. It has become apparent that saliva has many diagnostic uses especially in large scale screening. In the light of above context, this study was undertaken to evaluate salivary alterations by estimating the salivary glucose, total protein and albumin in T1DM and to compare with healthy nondiabetic controls. Further this study tends to highlight any significant correlation exists between serum and salivary glucose levels in T1DM.

MATERIALS AND METHODS

This study group comprised of randomly selected 30 diabetes patients with an already established diagnosis of T1DM, and a control of 30 healthy individuals. The T1DM and control group consisted of 16 males and 14 females, respectively. The patients included in the study were taken from District General Hospital Gulbarga and Rudrawadi’s Diabetic Care Center Gulbarga, with their prior consent.

Type 1 diabetes mellitus patients diagnosed by an endocrinologist through serum glucose test, age at diagnosis <15 years and on insulin therapy were included in T1DM group. Patients having systemic diseases other than DM were excluded from the study. The informed consent was obtained from both groups, and ethical clearance was obtained from Institutional Ethical Committee of Al-Badar Dental College; Gulbarga, Karnataka.

All the subjects included in the T1DM and control group were subjected to the following procedure:

- All the patients were advised not to eat anything, and until the samples were collected. The fasting blood and saliva samples from both study and control groups were collected between 8 and 10 a.m.

The unstimulated whole saliva was collected by spitting method. The patients involved in the study were asked to rinse the mouth thoroughly with water twice. Unstimulated whole saliva samples were collected at one sitting, during a period of 5 min. For collection of saliva, the subjects were asked to generate saliva in their mouths and to spit into sterile plastic container and stored over the ice. The collected saliva was subjected to glucose, total protein and albumin estimation. The patients were made to sit comfortably in a chair with arm extended straight from the shoulder. Blood sample was drawn from the antecubital vein. The collected blood was subjected to glucose estimation.

Methods of estimation

The serum and salivary glucose estimations were performed by glucose oxidase-peroxidase (GOD-POD) method, End point. The collected serum and salivary samples were centrifuged at 3000 rpm for 3 min. 5 µl sample was mixed with 500 µl of GOD-POD reagent and incubated at 37°C for 5 min. The glucose was estimated by placing the incubated samples in autoanalyzer, and the results were recorded.

The salivary total protein was performed by Biuret method, the peptide bonds of protein react with copper II ions in alkaline solution to form blue-violet complex, (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds, the color formed is proportional to the protein concentration and is measured at 546 nm (520-560 nm).

The salivary albumin estimations was performed by bromocresol green (BCG) dye method, albumin binds with BCG at pH 4.2 causing a shift in absorbance of the yellow BCG dye, blue-green color formed is proportional to the concentration of albumin present, when measured photometrically between 580 and 630 nm with maximum absorbance at 625 nm.

All estimations were performed using reagent kits of Transasia Bio-Medical Ltd., and on an Autoanalyzer (ERBA, Mannheim, Germany).

Statistical analysis

The values obtained for salivary glucose, total protein, albumin, and serum glucose were analyzed for mean and standard deviation (SD). Student’s t-test was employed to compare the values of T1DM and control group. The correlation between serum and salivary glucose in T1DM and control group was done by calculating Karl Pearson
correlation co-efficient, and the regression equation was calculated. All these statistical analyses were performed by using 11.5 version SPSS software, manufactured by SPSS Inc. [Chicago], and $P < 0.01$ was considered as significant.

**RESULTS**

According to the results of our study, the mean salivary glucose level was 0.813 mg/dl and SD 0.077 in the control group, whereas it was 2.12 mg/dl and SD 0.41 in T1DM group, which was having $t$ value of 16.37 and $P < 0.001$, which is statistically very highly significant (VHS). The mean serum glucose level was 82.96 mg/dl and SD 6.72 in the control group compared to 213.8 mg/dl and SD 39.0 in T1DM group, which was having $t$ value of 18.12, which is statistically VHS ($P < 0.001$) [Table 1].

The mean salivary total protein level in the control group was 134.13 mg/dl and SD 11.47 compared to 177.53 mg/dl and SD 9.03 in T1DM group, the $t$ value for the comparison of salivary total protein in T1DM and control group was $t = 16.31$ and $P < 0.001$, which connotes statistically VHS. The mean salivary albumin level in the control group was 59.0 mg/dl and SD 14.69 compared to 87.86 mg/dl and SD 6.54 in T1DM group, the $t$ value for the comparison of salivary albumin in T1DM and control group was $t = 9.85$ and $P < 0.001$, which is statistically VHS [Table 1].

There was a significant correlation observed between salivary and serum glucose levels in T1DM and control group. The Karl Pierson co-efficient for correlation between serum and salivary glucose in T1DM and control group were $r = 0.984$ and $r = 0.943$, respectively, whose $P < 0.01$. Both values showed a positive correlation between serum and salivary glucose, which is highly significant statistically [Graphs 1 and 2].

The regression co-efficient was calculated for the amount of increase or decrease in the serum glucose for a unit change in the salivary glucose. The salivary glucose was the independent variable and serum glucose was the dependent variable. Serum glucose can be predicted for a given value of salivary glucose using the regression equation for T1DM patients.

$$\text{Serum glucose} = 94.56 \text{ (salivary glucose)} + 13.37$$

The co-efficient of determination ($R^2$) for T1DM was 0.984 [Graph 2].

**DISCUSSION**

Saliva indeed is a mirror of our blood as these bio-fluids and their molecular components share many similarities. Realization of this fact and the possible utility of saliva as a diagnostic bio-fluid using recent technological advances over the past decades has enabled many researchers to develop saliva based technology to detect the transition between health and disease. As a diagnostic fluid, saliva offers distinctive advantages over serum because it can be

---

**Table 1: Comparison of serum glucose, salivary glucose, total protein, and albumin among the T1DM and control group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>T1DM group</th>
<th>Control group</th>
<th>$t$ test and $P$ value of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical details</td>
<td>Mean age 9.7±4.4 years</td>
<td>Age onset 6.8±3.2 years</td>
<td>Duration 5.4±2.8 years</td>
</tr>
<tr>
<td>Serum glucose</td>
<td>213.8±39.0</td>
<td>82.96±6.7</td>
<td>$t=18.1$ and $P&lt;0.001$ VHS</td>
</tr>
<tr>
<td>Salivary glucose</td>
<td>2.1±0.4</td>
<td>0.813±0.07</td>
<td>$t=16.3$ and $P&lt;0.001$ VHS</td>
</tr>
<tr>
<td>Salivary total protein</td>
<td>177.5±9.0</td>
<td>134.1±11.4</td>
<td>$t=16.3$ and $P&lt;0.001$ VHS</td>
</tr>
<tr>
<td>Salivary albumin</td>
<td>87.8±6.5</td>
<td>59.0±14.6</td>
<td>$t=9.8$ and $P&lt;0.001$ VHS</td>
</tr>
</tbody>
</table>

SD=Standard deviation, VHS=Very highly significant, T1DM: Type 1 diabetes mellitus
collected noninvasively by individuals with modest training. Furthermore, saliva may provide a cost effective approach for the screening of a large population.\[7\] It is beautifully said by Mandel that “saliva lacks the drama of blood, sincerity of sweat and emotional appearance of tears,” but still the fact is that, it is the vital element that sustains life in the oral cavity.\[13\]

Diabetes is broadly classified into T1DM and T2DM. T1DM is a severe disease that raises blood glucose concentration because of hyperglycemia and insulinopenia.\[14\] Diabetes can be detected by measuring the concentration of glucose in saliva; as glucose is present in the saliva of normal individuals.\[15\] The reasons for increased glucose content in the salivary secretion of diabetic patients has been explained by many authors. The salivary glands act as filters of blood glucose that are altered by hormonal or neural regulation.\[16\] The persistent hyperglycemia leads to microvascular changes in the blood vessels as well as basement membrane alteration in the salivary glands. This leads to increased leakage of glucose from the ductal cells of the salivary gland, thereby increasing the glucose content in saliva.\[17\-19\]

The salivary total protein is a vital component of saliva, predominantly comprising proline–rich proteins. The levels of proteins depend on the salivary flow rate, as the flow rate decreases the protein levels increases. Proteins found in saliva such as lactoferrin, lysozyme, peroxidase, defensins and histatins can destroy or inhibit the growth of microorganisms in the oral cavity. They can also maintain an ecological balance among the diverse bacteria that affect oral and general health.\[20\]

Albumin is the most osmotically active and abundant serum protein, accounting for \(> 50\%\) of all plasma proteins. Factors that regulate albumin synthesis are nutrition, hormonal balance and osmotic pressure.\[21\] In the oral cavity, albumin is regarded as a serum ultrafiltrate to the mouth, and it may diffuse into the mucosal secretions. Salivary albumin is increased in immunosuppression, diabetes and radiotherapy. It may be hypothesized that salivary albumin can be used to assess the integrity of mucosal function in the mouth. Albumin is often used as a marker for the degree of mucositis or inflammation in the oral cavity.\[9\]

A diagnosis and regular monitoring of diabetes is achieved by analyzing blood glucose levels, which is an invasive method and is physically and psychologically traumatic to the patient. Thus noninvasive, simple, and painless procedure such as salivary glucose estimation can be a desirable alternative.

The results of our study revealed that levels of salivary total protein were higher in T1DM group as compared to control group [Table 1]. These results are in accordance with the previous studies done by various authors.\[1\,16\,22\-26\] However, few studies showed no significant difference between the diabetic patients and controls.\[27\-29\] This contradiction can be explained by the fact that the studies might have incorporated different methodology and metabolic control status of the patients.

The salivary flow rate in diabetes is also diminished, which is related to salivary viscosity and foam. Viscosity otherwise called “spinbarkeit” and foam are reflected by the higher levels of proteins. Salivary turbidity is related to mucous, epithelial cells and especially to the presence of oral bacteria.\[16\]

The salivary albumin levels in our study were higher in T1DM group as compared with control group [Table 1]; these results are in concordance with the previous studies.\[1\,24\] However, few studies showed no change in salivary albumin levels in T1DM compared to controls.\[9\,27\,30\]

The elevated levels could be explained with abnormal binding of serum proteins to salivary gland basement membranes may be a reflection of increased permeability; this basement membrane permeability is often associated with diabetes, which leads to increased passage of proteins, IgG, albumin, and polyvalent immunoglobulins’ from exocrine glands into their secretions.\[29\,31\]

The results of our study showed that the salivary glucose levels in T1DM group were significantly higher compared to control group [Table 1]. These results are consistent with the studies done by various authors.\[16\,19\,25\-28\,32\-34\] The increased permeability of basement membrane in insulin-dependent DM may lead to enhanced leakage of serum-derived components into whole saliva through gingival crevices. The small glucose molecule can easily diffuse through the semipermeable basement membrane. Thus, a significant rise in glucose levels of saliva in patients with T1DM could be manifested.\[27\]

Further the results of our study showed highly significant positive correlation between serum and salivary glucose in T1DM patients, this finding is in agreement with the previous studies done by numerous authors.\[27\,32\-37\] Conversely a few authors have concluded that there is no correlation in contrary to our observation.\[1\,38\,39\] This conflict could be explained by the different type of saliva and methodology included in their study.

Regression co-efficient gives the amount of increase or decrease in the serum glucose for a unit change in the salivary glucose. According to our results serum glucose can be predicted for a given value of salivary glucose using the regression equation for T1DM patients (serum glucose = 94.56 [salivary glucose] + 13.37).

Although statistically salivary glucose is proved to be a good indicator for serum glucose levels, attention must once again
Salivary alterations in T1DM patients

Shahbaz, et al.

be drawn to the fact that glucose in whole saliva may not be entirely derived from the salivary glands. The results of our study will definitely add new dimensions and lay the foundation for further research and also making the estimation of glucose noninvasive.

CONCLUSION

There are definite changes in salivary composition, with increased levels of salivary glucose, total protein and albumin in T1DM patients compared to the healthy controls and further significant positive correlation was seen between the serum and salivary glucose levels in the T1DM patients. The levels of glucose in serum of T1DM patients could be reflected in saliva; hence the salivary glucose could be a marker for prediction of DM. In future, the estimation of glucose, large scale screening, and monitoring for diabetes could be noninvasive.

ACKNOWLEDGMENTS

The authors would like to thank the staff of District General Hospital and Rudrawadi’s Diabetic Care Centre, Gulbarga, for their support.

REFERENCES