Original Research

Evaluation of salivary glucose, amylase, and total protein in Type 2 diabetes mellitus patients


ABSTRACT

Background: Diabetes mellitus is a complex multisystem metabolic disorder characterized by a deficit in the production of insulin. The oral complications of uncontrolled diabetes mellitus are devastating. Saliva is an organic fluid that can be collected noninvasively and by individuals with limited training. These reasons create an interest in evaluating the possibility of using saliva as a diagnostic tool.

Aims and Objectives: The aim of this study was to determine, if saliva can be used as a noninvasive tool to monitor glycemic control in Type 2 diabetes. Comparative assessment of salivary (glucose, amylase, total protein levels) in patients with Type 2 diabetes and controls.

Materials and Methods: A total of 40 individuals, 20 with Type 2 diabetes and 20 controls of age group 40–60 years were selected for the study. Diabetic status was assessed by estimating random blood glucose levels. Unstimulated saliva was collected from each participant and investigated for glucose, amylase, and total protein levels. Salivary glucose estimation was performed using glucose-oxidase method, amylase by the direct substrate kinetic enzymatic method, and total protein by pyrogallol red dye end point method. All the parameters were subjected to statistical analysis using SPSS version 20.0.

Results: Significantly higher salivary glucose, lower amylase, and total proteins were observed in patients with Type 2 diabetes than controls. There was no significant correlation between salivary and blood glucose levels.

Conclusion: These results suggest that diabetes influences the composition of saliva. Since a significant correlation was not observed between salivary and blood glucose levels, further research is needed to determine salivary glucose estimation as a diagnostic tool for diabetes mellitus.

Key words: Diabetes mellitus, saliva, salivary amylase, salivary glucose, salivary total protein

387 million people were affected by diabetes worldwide and the number continues to increase. By 2035, it is estimated that 592 million might be affected by diabetes. India occupies the second position after the People’s Republic of China.[3]

Diabetes mellitus is one of the major health problems faced by the world today. Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin.[1] The prevalence of diabetes is rapidly rising all over the globe. It is estimated to be 2.8% in 2000 and 4.4% in 2030 worldwide.[2] Data indicates that in 2014, 187 million people were affected by diabetes worldwide and the number continues to increase. By 2035, it is estimated that 592 million might be affected by diabetes. India occupies the second position after the People’s Republic of China.[3]

Diabetes mellitus is classified based on etiology as Type 1, Type 2, other specific types, and gestational diabetes.[4] Type 2 diabetes mellitus is more common form of diabetes which is characterized by the dysfunction of β-cells to secrete adequate amounts of insulin, particularly after meals or peripheral insulin resistance.[5] Long-term systemic complications are microvascular disease, macrovascular disease, and neuropathy. Oral manifestations include gingivitis, periodontitis, xerostomia, salivary gland dysfunction, increased susceptibility to infections, caries, periapical abscesses, loss of teeth, impaired ability to wear dental prosthesis, taste impairment, lichen planus, and burning mouth syndrome.[6]
Currently, the diagnosis of diabetes is made only by analyzing blood glucose levels. These methods are invasive and are physically and psychologically traumatic to the patient. Hence, a noninvasive, simple, and painless procedure like salivary glucose estimation is very essential. Whole saliva contains locally produced substances and also serum components which can be used for the diagnosis of a variety of systemic diseases and understanding of their oral manifestations.

Among all salivary parameters glucose, amylase, and total proteins appear to be most closely related to the oral environment in patient with Type 2 diabetes. Glucose is a small molecule that diffuses through the membranes of blood vessels, passing from the blood plasma to the gingival fluid, through the gingival sulcus, and reaches the saliva. The increase in blood glucose in Type 2 diabetes patients patient can cause higher levels of salivary glucose with the consequent loss of homeostasis and greater susceptibility to disease in the oral cavity. Amylase plays an important role in the digestion of carbohydrates, which in turn acts as a potential factor in streptococcal adhesion to teeth and in plaque formation. Salivary total protein predominantly comprises of proline-rich proteins, mucin, amylase, immunoglobulins, statherins, and antibacterial factors which are responsible for most of the functions of saliva.

In India, the studies performed on the salivary composition of diabetic individuals are very few and the results reported so far were contradictory in several aspects which suggest the need for further investigative studies. The present study was conducted to assess variations in salivary glucose, amylase, and total proteins in a patient with Type 2 diabetes and to determine the role of saliva as a diagnostic tool.

MATERIALS AND METHODS

Source of data
The present study was carried out after the approval from the Institutional Review Board in the Department of Oral Pathology. Forty age and gender matched individuals, between 40 and 60 years were selected.

They were divided into two groups:
- Group I: Patients with Type 2 diabetes mellitus (n = 20)
- Group II: Control Group (n = 20).

Inclusion criteria
Patients visiting the outpatient department were included in the study:
- The selection of cases was done by the following criteria given by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus in 1998:
  - Symptoms of diabetes mellitus such as polyuria, polydypsia, polyphagia, and unexplained weight loss.
- Random non fasting plasma glucose levels ≥200 milligram per deciliter (mg/dl)
- Hemoglobin A1c levels were 6.5% or higher.
- Control group comprised of nondiabetic subjects without the symptoms of diabetes mellitus, blood glucose levels within normal limits (below <200 mg/dl), and hemoglobin A1c levels were between 4% and 5.6%.

Exclusion criteria
Patients with severe diabetic complications, other systemic illness, those using medication, pregnant women, smokers and alcoholics, persons treated with radiotherapy of the head and neck region, and persons with any oral lesions were excluded from the study.

The study protocol was explained and informed consent was obtained from the subjects. Thorough case history was taken followed by general and oral examination. Blood samples and unstimulated salivary samples were collected from each participant.

Methodology

Collection of blood samples
The subjects were made to sit comfortably on a chair with arm extended straight from the shoulder. The antecubital fossa was exposed and a tourniquet was applied about 1.5–2″ above the antecubital fossa. The area was rendered aseptic with cotton soaked in spirit. Using a 2 ml sterile disposable plastic syringe and a 24-gauge needle, the vein was punctured and 2 ml of blood was drawn. The tourniquet was relieved and cotton soaked with spirit was applied on the punctured site after the needle was removed. Blood was collected into ethylenediaminetetraacetic acid containing tube.

Serum glucose estimation
The levels were analyzed using glucose-oxidase method. The sample was centrifuged at 3000 rpm for about 5 min. One milliliter of glucose reagent was added to 10 µl of test sample and glucose standard. Both were incubated at 37°C for about 10 min. The absorbance values were measured on a semiautomatic analyzer and the values were expressed as mg/dl.

Collection of saliva
Patients were asked not to eat or drink 2 h before the time of saliva collection. Samples were collected 2 h after the subject’s breakfast. Spat technique was used to collect the unstimulated saliva. Salivary sample collection was performed in the morning between 9.00 and 11.00 am. Patient was asked to sit in the dental chair with head tilted forward and instructed not to speak, swallow or do any head movements during collection of the sample. Then the patient was instructed to spit the saliva in a sterile graduated container every minute for about 10 min. Saliva of about
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Salivary glucose estimation
Salivary glucose levels were measured using the glucose oxidase method in a semiautomated analyzer. The sample (100 µl) was mixed with the reagent in the ratio of 1:3 and incubated for 5 min at 37°C. The readings of absorbance values of standard and the sample against the reagent blank was noted. Standard was diluted 10 times for estimating salivary glucose levels. 14

Salivary amylase and total protein estimation
Salivary amylase levels were estimated using the direct substrate kinetic enzymatic method. Mean absorbance change per minute was calculated and expressed as units per liter. 15–17 Total protein estimation was done using pyrogallol red dye by the endpoint method. 18,19 Values were expressed as mg/dl.

Statistical analysis
Data entry, database management, and all statistical analysis were performed using Statistical Package for the Social Sciences (SPSS 20.0 version, Delaware, Chicago) software package. Values were expressed as means ± standard deviation and a P < 0.05 was considered significant. Comparison of blood glucose levels, salivary glucose, amylase, and total protein was done by unpaired t-test. Intragroup correlations were carried out by applying Karl Pearson’s correlation coefficient.

RESULTS
The study sample consisted of 40 patients, 20 in Group I (Type 2 diabetes mellitus) and 20 in Group II (control). In both the groups, 50% of patients were males and 50% females. The mean age of the study group was 50.38 ± 6.57 years. For each patient random blood glucose levels, salivary glucose, salivary amylase, salivary total protein levels were determined.

The mean random blood glucose levels in Group I (Type 2 diabetes mellitus) was 282.25 ± 42.81 mg/dl and in Group II (control) was 109.55 ± 11.19 mg/dl. The analysis was done using unpaired t-test, which showed a statistical significance with a P < 0.0001 [Table 1].

The mean salivary glucose level was significantly higher in Group I (8.45 ± 4.59 mg/dl) than in Group II (1.65 ± 0.30 mg/dl). The analysis was done using unpaired t-test, which showed a statistical significance with a P < 0.0001 [Table 2]. Mean salivary amylase levels were significantly lower in Group I (107.66 ± 28.60 U/ml) when compared to Group II (154.96 ± 25.07 U/ml) with a P < 0.0001 [Table 3]. The mean salivary total protein levels in Group I was 91.80 ± 6.61 mg/dl and in Group II (control) was 103.10 ± 5.46 mg/dl. A statistically significant difference was observed in both the groups with a P < 0.0001 and t = −5.8977 [Table 4].

Intragroup correlation was assessed using Karl Pearson correlation coefficient tests. There was no significant correlation between serum glucose levels and salivary glucose in Type 2 diabetes mellitus patients. Significant positive correlation was observed between salivary amylase and total protein levels. A positive correlation was also seen between salivary glucose levels and total protein levels [Table 5].

| Table 1: Comparison of type 2 diabetes mellitus and control groups with respect to blood glucose (mg/dl) by unpaired t-test |
|---|---|---|---|---|
| Group | n | Mean | SD | t value | P |
| Type 2 diabetes mellitus | 20 | 282.25 | 42.81 | 17.4550 | <0.0001* |
| Control | 20 | 109.55 | 11.19 |  |

*P<0.05, SD=Standard deviation

| Table 2: Comparison of type 2 diabetes mellitus and control groups with respect to salivary glucose (mg/dl) by unpaired t-test |
|---|---|---|---|---|
| Group | n | Mean | SD | t value | P |
| Type 2 diabetes mellitus | 20 | 8.45 | 4.59 | 6.6159 | <0.0001* |
| Control | 20 | 1.65 | 0.30 |  |

*P<0.05, SD=Standard deviation

| Table 3: Comparison of type 2 diabetes mellitus and control groups with respect to salivary amylase (U/ml) by unpaired t-test |
|---|---|---|---|---|
| Group | n | Mean | SD | t value | P |
| Type 2 diabetes mellitus | 20 | 107.66 | 28.60 | −5.5617 | <0.0001* |
| Control | 20 | 154.96 | 25.07 |  |

*P<0.05, SD=Standard deviation

| Table 4: Comparison of type 2 diabetes mellitus and control groups with respect to salivary total protein (mg/dl) by unpaired t-test |
|---|---|---|---|---|
| Group | n | Mean | SD | t value | P |
| Type 2 diabetes mellitus | 20 | 91.80 | 6.61 | −5.8977 | <0.0001* |
| Control | 20 | 103.10 | 5.46 |  |

*P<0.05, SD=Standard deviation

| Table 5: Correlation coefficient among blood glucose (mg/dl), salivary glucose (mg/dl), salivary amylase (U/ml), and salivary total protein (mg/dl) in type 2 diabetes mellitus group by Karl Pearson’s correlation coefficient |
|---|---|---|---|---|
| Variables | Blood glucose (mg/dl) | Salivary glucose (mg/dl) | Salivary amylase (U/ml) | Salivary total protein (mg/dl) |
| Blood glucose (mg/dl) | 1.0000 |  |  |  |
| Salivary glucose (mg/dl) | 0.1350 | 1.0000 |  |  |
| Salivary amylase (U/ml) | 0.3098 | 0.3328 | 1.0000 |  |
| Salivary total protein (mg/dl) | 0.2408 | 0.5181 | 0.4842 |  |

*P<0.05
DISCUSSION

Diabetes mellitus is a group of chronic diseases characterized by insulin deficiency, cellular resistance to insulin action, or both which results in hyperglycemia, and other related metabolic disturbances. It is associated with serious complications of the eyes, kidneys, heart and blood vessels, and other organ systems which may impair quality of life and shorten the patient’s lifespan.[30]

The use of saliva for diagnosis is recently on increasing trend rather than blood. Saliva as a diagnostic tool has some distinct advantages. It can be collected noninvasively, and by individuals with limited training, no special equipment is needed for collection. Diagnosis of disease based on the salivary analysis is potentially valuable for children and older adults, since the collection of fluid is associated with fewer compliance problems when compared with a collection of blood.[7]

In the present study, we observed that the concentration of salivary glucose in Type 2 patient with Type 2 diabetes was significantly higher than in control group. This is in accordance with the results obtained by Carda et al. in 2006, Aydin in 2007, Sashikumar and Kannan in 2010, Vasconcelos et al. in 2010, Panchbhai et al. in 2010.[8,10,14,21,22] However, there is a difference in the mean salivary glucose levels, and this can be due to differences in methods used for glucose estimation, saliva collection, and dietary pattern. The elevated salivary glucose levels are due to diabetic membranopathy, which leads to leakage across the basement membrane and raised percolation of glucose from blood to saliva.[9,23]

The significantly lower mean salivary amylase levels in patients with Type 2 diabetes when compared to controls observed in our study is in agreement with studies by Yavuzyilmaz et al. in 1996 and Panchbhai et al. in 2010.[8,24] This is because of the hormonal and metabolic changes in diabetic patients.

The present study showed significant lower mean salivary total protein levels in patients with Type 2 diabetes. Similar results were obtained by Streckfus et al. in 1994.[25] This is due to protein utilization by other metabolic pathways, a systemic response to glucose intolerance.

There was no significant correlation between serum and salivary glucose levels in patients with Type 2 diabetes which is in accordance with Forbat et al. in 1981, Carda et al. in 2006, Jurysta et al. in 2009 studies.[21,26,27]

A significant positive correlation was seen between salivary protein levels and serum glucose levels in Type 2 diabetes patients in the present study as reported previously by Panchbhai et al. in 2010 in the controlled diabetic group.[8]

The present study showed significance between salivary amylase levels and total protein levels in patients with Type 2 diabetes. Similar results were observed in studies by Pal et al. in 2003 and Panchbhai et al. in 2010.[8,28] The reason is salivary amylase is a component of total protein and any variations in amylase will simultaneously affect total protein levels.

CONCLUSION

The results of the present study suggest that in Type 2 diabetes patients, the constituents of saliva such as salivary glucose, salivary amylase, and salivary total protein levels are altered. Since a significant correlation was not observed between salivary and blood glucose levels, further studies should be carried out on larger population and in different areas in order to establish salivary glucose estimation as a diagnostic and monitoring tool for diabetes mellitus.

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