Original Research Article

Study of salivary α-amylase immunoglobulin, a and flow rate in diabetic subjects: a cross sectional study

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ABSTRACT

Background: Diabetes is possibly the mainly common metabolic syndrome with salivary inference. However, lack of literature on the possible relationship between diabetes and salivary α-amylase, Immunoglobulin-A (IgA) and flow rate. Therefore, present study aims to estimate of salivary α-amylase, immunoglobulin-A and flow rate in diabetic subjects of Indian population.

Methods: The present cross sectional study was done on 60 subjects of diabetes mellitus (T2DM) and 60 subjects’ non-diabetic healthy controls at Banas medical college and our hospital. The subject’s demographic and anthropometric parameters were recorded; detailed history and clinical examination were performed in the entire cases. The un-stimulated saliva was collected in the fasting state. Salivary flow rate, biochemical variables and IgA were analyzed. Data which was collected was statistically analyzed.

Results: The results showed that salivary glucose (p<0.001) and urea levels (p<0.001) were significantly higher in diabetic patients compared with non-diabetic subjects. It was also found that the diabetic patients had significant reduction in salivary flow rate (p<0.01), α- amylase (p<0.001) and total protein levels (p<0.05) when compared with non-diabetic individuals. In contrast, there was no significant difference was observe in levels of IgA (p>0.05) between diabetic and non-diabetic subjects.

Conclusions: In our study, we conclude that significant differences were reported in salivary flow rate, α-amylase and IgA between diabetics and non diabetics. Therefore, we suggested that estimation of salivary parameters might be a cost effective and a non invasive choice for screening, diagnosis and monitoring of diabetes instead of blood.

Keywords: Diabetes mellitus, Salivary α-amylase, IgA, Salivary flow rate, Salivary protein

INTRODUCTION

Diabetes is an extensive metabolic syndrome sourcing well-documented harmful effects on the physical condition of person. Various epidemiologic researches have recommended that diabetes is jeopardy for the increase of oral diseases in humans. Diabetes is possibly the mainly common metabolic syndrome with salivary inference. About one third of diabetic patients grumble of dry mouth (xerostomia) which may be due to overall decreased flow of saliva ensuing from systemic dehydration and an augment in the salivary glucose level. Salivary glands under functions and enlarged vulnerability to oral infections such as caries or periodontitis have long been known sign and symptoms of diabetes mellitus, mainly when there has been dehydration and insufficient blood glucose maintained. The understanding of the responsibility of each salivary constituent in the oral cavity homeostasis is vital to recognize how its changes or absence may be associated with pathological circumstances. The most frequently used laboratory diagnostic measures engage the
investigation of the cellular and chemical components of blood. Other biological fluids are utilized for the diagnosis of disease, and saliva suggests a little distinguishing benefit. In addition, it has been illustrated that the salivary glands are also pretentious directly or circuitously. Data on oral health complications which are linked with T2DM, which are frequently came across by physicians embrace xerostomia, tooth loss, gingivitis, periodontitis, odontogenic abscesses and soft tissue lesions of the tongue and the oral mucosa. Numerous physiologic features participate to compromised salivary function in inadequately controlled T2DM. It has been associated with autonomic neuropathies, microvascular changes; hormonal imbalances and its amalgamation of these are accountable for salivary under function and dehydration in diabetics patients. Biochemical investigation of saliva would be of vast biomedical significance, since saliva is quite easy to collect and contribution a cost-effective approach for screening of great populations, and could symbolize a substitute for the patient whose blood is not easy to obtain when obedience is a trouble. However, lack of literature on the possible relationship between diabetes and salivary α-amylase and flow rate in Indian populations. Thus, the present study is undertaken to identify the impact of diabetes mellitus on salivary α-amylase, immunoglobulin-A and flow rate in Indian populations.

METHODS

The present cross sectional study was carried out in the department of dentistry, general hospitals associated with Banas medical college and research institute Palanpur and our private dental clinic, Banaskantha, Gujarat, India, over period of six months from July 2021 to December 2021. All type 2 diabetes mellitus patients attending the OPD of the hospital during the study period were enrolled in the present study. Total 60 type 2 diabetes mellitus patients, age ranging 35 to 50 years, were selected for present study. 60 age and sex-matched healthy volunteers selected from the patient’s entourage and health care professionals were incorporated in the control group. Subjects with any acute infirmity, any acute or complex chronic complications of diabetes mellitus were excluded from present study. The written informed consent was obtained from all participants before starts of study.

Demographic information was collected from all subjects by semi structured questionnaire. A detailed clinical history was taken such as age and sex, symptomatic, past history of hypertension and other endocrine disorders, any family history of diabetes, hypertension, dyslipidemia, liver disease, history of smoking, and history of alcohol consumption. Clinical examination was also performed, anthropometric measurement including height, weight, BMI, waist circumference, hip circumference, and waist to hip ratio were also measured. The waist circumference was measured at the mid-point between the lower border of the rib cage and the iliac crest, whereas the hip circumference was recorded at the widest point between the hip and buttock.

The saliva was collected from all subjects in the morning between 6 am to 8:00 am in the fasting state. Unstimulated entire saliva was collected in vial by standardized spitting method, for 4-5 minutes. Salivary flow rate was measured and it was showed as milliliters per minute. All salivary samples were transported within half an hour to diagnostic laboratory. All salivary samples were centrifuged at 4000 rpm for 4-5 minutes and the supernatants were collected and they were stored at -40 to 80 °C until further investigation. Biochemical parameters like salivary glucose, salivary α-amylase, urea and total protein were analyzed using commercial available diagnostic kit by using a semi auto analyzer. A salivary total IgA antibody was estimated by enzyme-linked immunosorbent assay (ELISA) by using with as commercial available diagnostic kit (anti-human IgA - Sigma, St. Louis, USA). The study protocol was approved by institutional ethics committee human (IEC-H).

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences, version 20 (SPSS Inc., Chicago, IL). Results for continuous variables are presented as mean ± standard deviation, and unpaired student’s test was used for significant difference between two variables. Chi-square test and Fisher’s exact chi Square test were used for the comparison of categorical variables and presented as percentage. The diagnostic accuracy of salivary variables in diabetes mellitus subjects was assessed by receiver operating characteristic (ROC) curve analysis. The level p<0.05 was considered as significance.

RESULTS

Demographic characteristics are presented in (Table 1-2). The total of 120 subjects was included in this study. Total 60 subjects with diabetes mellitus 2 type (20 female, 40 male, mean age 41.63±4.30) included in study group and control group included 60 subjects (25 female and 35 male, mean age 39.98±2.32). Total male subjects are 75 (62.5%) and female subjects are 45 (37.5%) in both groups. In our study, preponderance of the T2DM subjects was in the age group of 41-45 years. Out of 60 subjects, 10 subjects are 35-40 years (16.66%), 30 subjects are 41-45 years (50%), 20 subjects are 46-50 years (33.33%). The anthropometric and salivary glands variables in the study population are shown in (Table 3-4). Differences between anthropometric and salivary glands variables like amylase, flow rate and IgA between Diabetic Subjects (T2DM) and Non-Diabetic subjects were tested by Student independent t-test. Mean value for (p<0.05), body mass index (p<0.01), waist-hip ratio (p<0.05), salivary urea levels (p<0.001) and glucose levels (p<0.001) were significantly higher in the T2DM subjects as compared to non-diabetic subjects where as
saliary flow rate (p<0.01), total protein (p<0.05) and saliary α-amylase level (p<0.001) were significantly decreased in the T2DM subjects when compared to non-diabetic Subjects. There was no significant differences (p>0.05) was observed in regards to IgA in both diabetic and non-diabetic subjects. Flow rate was significantly diminished in diabetics. A ROC curve was build to validate the salivary variables that might be used for diagnostic testing. Glucose, total protein, urea, total IgA, α-amylase and salivary flow rate were incorporated in the investigation and all are statistically significant (Table 5).

Table 1: Age wise distribution of subjects in both groups (n=102).

| Age group (years) | Diabetic subjects N (%) | Non-diabetic subjects N (%) | Level of significance |
|-------------------|-------------------------|----------------------------|----------------------|
| 35-40             | 10 (16.66)              | 20 (33.33)                 | p<0.05               |
| 41-45             | 30 (50)                 | 30 (50)                    |                      |
| 46-50             | 20 (33.33)              | 10 (16.66)                 |                      |
| Total             | 60 (100)                | 60 (100)                   |                      |

Table 2: Distribution of subjects according to gender.

| Gender          | Diabetic subjects N (%) | Non-diabetic subjects N (%) | Level of significance |
|-----------------|-------------------------|----------------------------|----------------------|
| Male            | 40 (66.66)              | 35 (58.33)                 | p<0.05               |
| Female          | 20 (33.33)              | 25 (41.66)                 |                      |
| Total           | 60 (100)                | 60 (100)                   |                      |

Table 3: Anthropometric variables in the study participants.

| Variables Studied | Non-Diabetic Subjects (Mean±SD) | Diabetic Subjects (Mean±SD) | Level of significance |
|-------------------|---------------------------------|-----------------------------|----------------------|
| Age (years)       | 39.98±2.32                      | 41.63±4.30                  | p>0.05               |
| BMI (kg/m²)       | 22.67±1.76                      | 28.43±2.31                  | p>0.01               |
| Waist-hip ratio   | 0.89± 0.01                      | 0.97±0.03                   | p<0.05               |

DISCUSSION

In our study, the salivary glucose level of diabetic patients was found to be significantly higher than that of non-diabetic patients; in reliable with previous results.10-12 The high salivary glucose level is an outcome of high plasma glucose level from which saliva is formed. This high salivary glucose in concurrence with overall decreased flow of saliva has also been reported to be accountable for the grumble of dry mouth by the diabetic patients.6 In addition; the high glucose level in saliva of diabetic patients might contribute to their vulnerability to oral infections like periodontal disease and dental caries.13 The hyperglycemic atmosphere can decrease tissue growth and matrix synthesis by fibroblasts and osteoclasts. As a result, the tissues are weaker and wound healing is delayed.14

Table 4: Salivary biochemical, immunological and flow rate in diabetic and non diabetic subjects.

| Variables studied | Non-diabetic subjects (Mean±SD) | Diabetic subjects (Mean±SD) | Level of significance |
|-------------------|---------------------------------|-----------------------------|----------------------|
| Salivary flow rate ml/min | 0.76±0.4 | 0.42±0.04 | p<0.01 |
| Glucose (mg/dl)    | 5.10±0.52 | 19.43±3.89 | p<0.001 |
| Total protein (mg/dl) | 2.58±0.76 | 1.05±0.52 | p<0.05 |
| Salivary α-amylase (AU/dl) | 85.41±12.64 | 17.65±1.86 | p<0.001 |
| Urea (mg/dl)       | 16.21±2.14 | 29.54±3.13 | p<0.001 |
| Total IgA           | 35.11±56 | 36.41±25 | p>0.05 |

Table 5: Area under the receiver operator characteristic (ROC) curve obtained for salivary variables of diabetic patients.

| Salivary variables | Area under the ROC curve | P value |
|--------------------|--------------------------|--------|
| Glucose            | 0.97                     | <0.001 |
| Total protein      | 0.96                     | <0.001 |
| Salivary α-amylase | 0.98                     | <0.001 |
| Urea               | 0.84                     | <0.001 |
| Total IgA          | 0.87                     | <0.001 |

The results of our study showed a significantly reduced salivary flow rates in diabetic patients when compared with non-diabetic individuals. A similar finding was also reported by Meurman and Dodds suggesting the presence of diabetes-induced decline of salivary gland function.15-16 Analogous findings have also been earlier explained in the literature and were linked with diabetes-induced-neuropathic changes in the salivary parenchyma with lymphocytic gland infiltrate similar to the one occurring in the pancreas of these diabetic patients.17 The reduced salivary flow rates have been reported to be more common in uncontrolled diabetic patients.18 Total amylase and protein were significantly lower in diabetic patients. Amylase was evaluated as a marker of metabolic
and hormonal changes in salivary gland products. Yavuzyilmaz also reported lower levels of amylase in diabetic patients. In contrast, salivary total protein concentration differed from that reported in studies evaluating this parameter after freezing and storage of the sample. This difference might be explained by the fact that in the present study the assay was performed instantly after collection and centrifugation of the saliva samples to prevent endogenous proteolytic activity. In this study, evaluation of immunological changes in saliva showed a higher total IgA antibody concentration in diabetic patients. Similar results have been reported in other studies evaluating the salivary production of IgA antibodies in diabetic patients.

Biochemical and immunological parameters modifications in the saliva of diabetic patients have been explained and salivary alter may act as a balancing components for the diagnosis of diabetes mellitus. In the present study, the clinical presentation of an individual test was established by the ROC curve after the variables had been unitized and were validated for diagnostic purpose. The larger the capacity of a test to differentiate between diabetic and non-diabetic subjects, the nearer is the curve to the left corner of the chart, with an area under the curve close to one. The series of individual variables that best distinguish diabetic patients was glucose (area of 0.97), total protein (area of 0.96), amylase (area of 0.98), IgA (area of 0.87), and urea (area of 0.84). The accurateness of the diagnostic test combining these parameters was 84%. A similar accuracy (85%) has been reported by Lopez et al who used saliva for the diagnosis of diabetes in children considering calcium, urea, total sugars, glucose and total protein concentration as done in the present study for adult patients.

Entire saliva can be collected non-invasively and by persons with inadequate training. No particular equipment is required for its compilation. Diagnosis of a disease by doing the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is related with fewer conformity evils in comparison to the collection of blood. Furthermore, the analysis of saliva seems to be a cost-effective move toward in the screening of huge populations.

Limitations

The present study has some limitations such as little sample size which vetoed to present study from arriving at conclusions on the changed in salivary variables in diabetic subjects. A bigger sample size would have facilitated to our study in establishing the association between fasting plasma glucose levels and a variety of salivary variables. In addition, HbA1c was not estimated in this study thus it would have been a value addition if the status of the glycaemic controls of the subjects it had been analyzed.

CONCLUSION

In our study, we conclude that a combination of immunological (IgA) and biochemical variables as constructive markers to supervise diabetic patients, because significant differences were observed in the entire saliva composition of diabetic patients, the changes noticed demonstrating a sturdy association between our outcomes and the systemic health condition of the patient. Furthermore, some salivary variables were reported to be helpful to categorize an adult as diabetic. These differences in salivary composition therefore recommend the use of saliva as a substitute fluid to observe patients with diabetes mellitus, allowing for the raise of diabetes not only in Indian but also in other countries or worldwide.

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REFERENCES

1. Nechifor M, Teslariu E, Mindreci I. The influence of Magnesium, chromium and copper in alloxan-induced diabetic rats. Adv Mag Res Nutr Health. 2001;2(4):56-9.
2. Cianciola LJ, Park BH, Bruck L, Mosovich I, Genco RG. Prevalence of periodontal disease in insulin dependent diabetes mellitus. J Am Dent Assoc. 1982;104:653-60.
3. Manfredi M, McCullough M, J, Vescovi P, Al-Arawi ZM, Porter SR. Update on diabetes Mellitus and related oral diseases. Oral Dis. 2004;10:187-200.
4. Mata AD, Marques D, Rocha S, Francisco H, Santos C, Mesquita MF, Singh J. Effects of diabetes mellitus on salivary secretion and its composition in human. Molecular and cellular Biochemistry. 2004;2:137-42.
5. Sreebny LM, Yu A, Green A, Valdini A, Xerostomia in diabetes Mellitus. Diabetes care. 1992;15:900-4.
6. Twetman S, Johansson I, Birkhed D, Nederfors T. Salivary fuction and glycemic control and caries associated risk factors. Caries Res. 2002;36(1):312-35.
7. Chavez M, Borrell LN, Taylor GW and ship JA. Salivary function and glyceric control in older persons with diabetes. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2000;89:305-11.
8. Mandel ID. Salivary diagnosis: more than a lick and a promise. JAM Dent Assoc. 1993;2:85-7.
9. Kaufman E, Lamster IB. The diagnostic applications of saliva a review. Crit Rev Oral Biol Med. 2002;13:197-212.
10. Ben-Aryeh H, Serouya R, Kanter Y, Szargel R, Lauer D. Oral health and salivary composition in diabetic patients. J Diab Complicat. 1993;7(1):57-62.
11. Ayadin S. Comparison of ghrelin, glucose, alpha amylose and protein levels in saliva from diabetics. J Biochem Molecular Biol. 2007;40:29-35.
12. Vasconcelos AC, Soares MM, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. J Oral Science. 2010;52:293-8.
13. Tervonen T, Kunnutila M. Relation of diabetes control to periodontal pocketing and alveolar bone level. Oral Surg Oral Med Oral Pathol. 1986;64:346-9.
14. Rammamuthy NS and Golub I.M. Diabetes increases collagenase activity in extracts of rat gingiva and skin. J Perio Research. 1983;18:23-30.
15. Meurman JH, Collin HL, Niskanen L. Saliva in non-insulin dependent diabetic patients and control subjects: the role of autonomic nervous system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;86:69-76.
16. Dodds MW and Dodds AP. Effects of glycemic control on saliva flow rates and protein composition in non insulin dependent diabetes mellitus. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998;83(4):465-70.
17. Markopoulos AK, Belaxi M. Histopathological and immunohistochemical features of labial salivary glands in children with type 1 diabetes. J Diab Complicat. 1998;12:39-42.
18. Ship JA, Pillemer SR, Baum BJ. Xerostomia and the geriatric patient. J Am Geriatr Soc. 2002;50(3):535-43.
19. Yavuzyilmaz E, Yumar O, Akdoganli T, Yamalik N, Ozer N, Ersoy F, Yenia YI. The alterations of whole saliva constituents in patients with diabetes mellitus. Aust Dent J. 1996;41:193-7.
20. Flegal KM, Ezzati TM, Harris MI, Haynes SA, Juarez RZ, Knowler WC. Prevalence of diabetes in Mexican Americans, Cubans, and Puerto Ricans from the Hispanic health and nutrition survey, 1982–1984. Diab Care. 1991;14:628-38.
21. Bigler LR, Streckfus CF, Dubinsky WP. Salivary biomarkers for detection of malignant tumors that is remote from oral cavity. Clin Lab Med. 2009;29:71-85.
22. Batista JE, Batista CEM, Monteiro Neto V, Guerra RNM. Colonization by Streptococcusmutane anti-insulin antibody dosages in the saliva of diabetics. Rev Cienc Saude. 2004;6:11-20.
23. Moura SAB, Medeiros AMC, Costa FRH, Moraes PH, Oliveira filho SA. Diagnostic value of saliva in oral and systemic diseases: a literature review. Pesq Bras Odontop Clin Integr. 2007;7:187-94.
24. Martinez EZ, Louzada-Neto F, Pereira BB. The ROC curve for diagnostic tests. Public Health Notebooks. 2003;11:7-31.
25. Lopez ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Braz Dent J. 2003;14:26-31.

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