

Estimation of Salivary Amylase in Diabetic Patients and Saliva as a Diagnostic Tool in Early Diabetic Patients

L. MALATHI¹, K.M.K. MASTHAN², N. BALACHANDER³, N. ARAVINDHA BABU⁴, E. RAJESH⁵

ABSTRACT

Aim: The aim of this study was to estimate the salivary amylase levels in non-insulin dependent diabetes mellitus patients and to correlate these findings with those in normal individuals, in order to provide salivary amylase level as a bio-chemical indicator for diagnosing and monitoring the glucose levels.

Material and Methods: The study samples consisted of 60 individuals. Both males and females participated in the study. Thirty non-insulin dependent diabetes mellitus patients of age group of 30 to 60 years and healthy individuals of same number and age group were included in this study. The data obtained in this study were statistically analyzed by using Student's t-test.

Results: In estimation of salivary amylase levels, the comparison of mean and standard deviation showed the highest mean score (2739.48 ± 1525.20) among the diabetic patients and lowest mean score (1740.38 ± 638.51) among the non-diabetic patients.

The p-value obtained was less than 0.01. Hence, a highly significant difference in the mean scores regarding salivary amylase (u/l) was found among the two groups.

Conclusion: The mean scores of age, fasting blood sugar, post prandial blood sugar, HbA1c and salivary amylase levels were greater in diabetic patients than in non-diabetic patients.

Keywords: Diabetes mellitus, Saliva, Diagnosis, Salivary amylase, Blood sugar

INTRODUCTION

Diabetes mellitus is an iceberg disease [1]. Diabetes mellitus is 6th leading cause of death worldwide and it is considered to be a complex disease. It is associated with many oral complications such as gingivitis, periodontitis, dental caries, dental abscesses, xerostomia and certain fungal infections, in addition to nephropathy, retinopathy, neuropathy, and cardiovascular diseases [2].

We conducted this study to estimate salivary amylase levels in non-insulin dependent diabetes mellitus patients. With the results which we obtained, we wanted to analyze the changes in salivary amylase levels in non diabetic and diabetic individuals and possibility of using salivary amylase as an indicator of increased glucose levels.

MATERIAL AND METHODS

This study was conducted at Sree Balaji Dental College and Hospital in association with Bio-Line Laboratory, Chennai, India. The study samples consisted of 60 individuals who included both males and females. Non-insulin dependent diabetes mellitus patients and the same number of healthy individuals of the age group of 30-60 years were included. All patients were from different socio-economic status.

Ethical Clearance

Ethical clearance was obtained from the institutional ethical committee, Chennai, before the study was started. The nature and purpose of the study were briefly explained to each participating patient/subject and their written consent was obtained.

Inclusion Criteria

Patients diagnosed as diabetic by medical faculty, who had elevated blood glucose levels, were included. The second group consisted of non-diabetic individuals.

Exclusion Criteria

Patients having severe diabetic complications, other systemic illness and those who were under medication for other diseases, were excluded from this study.

Clinical Examination

General and oral examinations of the patients and control subjects were recorded and their case histories were taken.

During the clinical examination, the following elements were analyzed.

- Gingival status
- Periodontal condition
- Xerostomia
- Dental caries

Collection of Saliva

Salivary samples were collected in the morning. The diurnal variation of saliva is very low during this time. After making the subjects rinse their mouths thoroughly with water, salivary samples were collected in sterile containers by instructing them to allow saliva to collect naturally in mouth and to expectorate it, into the containers. The samples were immediately taken for biochemical analysis.

Collection of Blood

Antecubital venous blood samples were taken after 12hrs of overnight fasting. The collected samples are used for the estimation of blood glucose and HbA1c levels. The estimation of blood glucose was done by GOD-POD method.

Estimation of salivary amylase activity was determined by the direct substrate method (kinetic enzyme assay). The ability of alpha amylase to catalyze the hydrolysis of starch to maltose is the

principle used to estimate amylase. The saliva was collected, it was diluted to 1 in 100 (1 part of saliva and 99 parts of saline) and it was added to the reagent (ready to use liquid kit). The sample (saliva) reagent was placed in a sample cap and it was analyzed in an automatic analyzer.

RESULTS

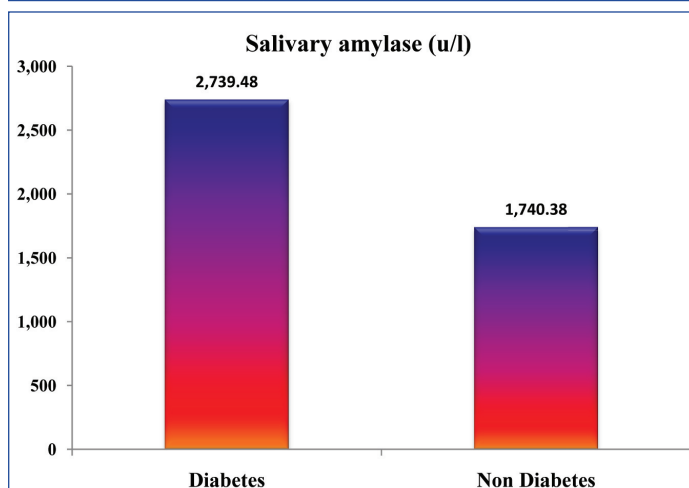
The significant differences between the mean scores regarding the age, fasting blood sugar (mg/dl), post prandial blood sugar (mg/dl), HbA1c(%) and salivary amylase (u/l) were analyzed using the inferential statistics of Student's t-test, after verifying the normality assumption by Q-Q plot technique. The results have been given in [Table/Fig-1].

The fasting blood sugar, postprandial blood sugar and HbA_{1c} of the diabetic patients in our study showed highest mean scores and the p value which was obtained was less than 0.05.

The salivary amylase estimation showed highest mean scores among the diabetic patients than among the non-diabetic patients in our study and the p value was less than 0.05 [Table/Fig-2].

	Group	n	Mean	SD	SE	t	p
Age (yrs)	Diabetes	33	47.21	7.817	1.36	0.412	0.682
	Non Diabetes	34	46.44	7.500	1.29		
Fasting blood sugar (mg/dl)	Diabetes	33	173.88	72.02	12.54	6.79	< 0.00**
	Non Diabetes	34	89.21	9.84	1.69		
Post-prandial blood sugar (mg/dl)	Diabetes	33	247.88	86.37	15.04	3.47	0.001**
	Non Diabetes	34	141.62	154.08	26.42		
HbA _{1c} (%)	Diabetes	33	7.79	1.15	0.20	11.84	< 0.00**
	Non Diabetes	34	5.15	0.60	0.10		
Salivary amylase (u/l)	Diabetes	33	2,739.48	1,525.20	265.50	3.52	0.001**
	Non Diabetes	34	1,740.38	638.51	109.50		

[Table/Fig-1]: Test for Mean score analysis w.r.t group of patients
** Highly Significant (Sig. at 1%)



[Table/Fig-2]: Mean score of salivary amylase among diabetic and non-diabetic patients

The selected parameters were estimated and means \pm SD of the various parameters in non-insulin dependent diabetic patients are as follows:

Variables	Mean (\pm SD)
Fasting blood glucose	0.970 mg/dl
Post-prandial blood glucose	0.881 mg/dl
Salivary Amylase	0.767 u/l
HbA _{1c}	0.467%

- Mean blood glucose in diabetic individuals was significantly higher than that in control group.
- Mean salivary amylase in diabetic individuals was significantly higher than that in control group.

- Mean HbA_{1c} in diabetic individuals was significantly higher than that in control group.
- In diabetic patients, there is a significant linear relationship between blood glucose and salivary amylase.

DISCUSSION

Diabetes is a genetically and clinically heterogeneous group of disorders that share glucose intolerance in common.

The present study was conducted to estimate the salivary amylase levels in non- insulin dependent diabetes mellitus patients and to compare the values with those of normal individuals, to use salivary amylase as a bio-chemical indicator to diagnose diabetes mellitus and also to monitor such patients.

The oral findings of 30 non-insulin dependent diabetic patients revealed 7 patients with poor oral hygiene and halitosis and 12 patients showed periodontitis. Paresthesia, xerostomia and burning sensation were presented in 2 patients and 1 patient had oral Candidiasis. Rest of the patients showed mild to moderate gingivitis.

Xerostomia (dry mouth) occurring in diabetic patients can be multifactorial, either due to infiltration of fat cells into the salivary glands or physical alteration of mucosal cells, subsequent to dehydration caused by polyuria or microvascular disease, local inflammation and irritation in the oral cavity, infections, metabolic disturbances and neuropathy affecting salivary glands and it may also be caused by drug therapy given for diabetes. The finding of xerostomia was consistent with the findings of studies conducted by She-Fen Chaung et al., [3], Busato IM et al., [4], Paul A.Moore et al., [5].

A significant increase in salivary amylase levels among diabetic patient in our study group was consistent with the findings of previous studies done by Prabal Pal et al., [6]. Our study correlated with their study in significance level of $p < 0.01$.

Sathyapriya et al., [7], in their research done on the potential of salivary protein as a biomarker in prognosis of diabetes mellitus, found that mean salivary glucose levels were higher in the uncontrolled and controlled diabetic groups than in the healthy non-diabetic group and that the differences were highly significant.

The salivary amylase levels were significantly higher in controlled diabetics as compared to those in healthy non-diabetics. Our study correlated with their study in significance levels of $p < 0.05$.

María Elena Lopez et al., [8], in their study done on salivary characteristics of diabetic children, gave the inference that salivary protein and salivary amylase increased in diabetics than in controls.

Prabal Pal et al., [7], in their study done on estimation of salivary glucose, salivary amylase, salivary total protein and periodontal micro flora in saliva of diabetic patients, concluded that salivary amylase caused increased accumulation of plaque in the interdental areas and that it increased concentration of salivary glucose in diabetic individuals. It also favoured proliferation of both aerobic and anaerobic bacteria in the gingival plaque of diabetic individuals.

Scannapieco et al., [9], in their study, showed that increased salivary amylase promoted adhesion of oral Streptococci to hydroxyapatite, thus supporting the role of salivary amylase as a potential factor in Streptococcal adhesion to teeth and in plaque formation.

Decrease in salivary flow rate or oral dryness occurring in diabetes can be multifactorial, either due to infiltration of fat cells into the salivary glands or due to physical alteration of mucosal cells, subsequent to dehydration caused by polyuria or microvascular disease, local inflammation and irritation in the oral cavity, infections, metabolic disturbances and neuropathy affecting salivary glands and it may also be caused by drug therapy given for diabetes.

Arati S. Panchbhai et al., [10], Prabal Pal et al., [7], Conner S. et al., [11], Straka M. [12] and Hanna-Leena Collin [13] had mentioned

increased caries activity in diabetic patients, which was also seen in our study.

In our study, the poor glycemic control determined by the HbA1c values also indicated increased salivary glucose and salivary amylase levels.

CONCLUSION

The present study included critical salivary parameters, namely fasting, post prandial salivary amylase and HbA1c levels, in order to study the effects of diabetes on salivary composition. The study also gave due consideration to the levels of metabolic control, duration and type of diabetes mellitus and gender. In addition, the study focussed on most possible interrelationships among the variables.

Like our study, several studies have confirmed that salivary amylase caused increased accumulation of plaque in the interdental areas. Increased concentration of salivary glucose in diabetic individuals also favoured proliferation of both aerobic and anaerobic bacteria in the gingival plaque of diabetic individuals.

As in various studies quoted previously, our study also showed significant increase in the salivary amylase levels in non-insulin dependent diabetic patients. Thus, our study is one such evidence which has been added to previous studies in supporting the use of salivary amylase estimation as a non-invasive procedure for diagnosing and monitoring the blood glucose levels. The present study had a small sample size, but it made some novel observations which will unquestionably provide a platform for further research.

REFERENCES

- [1] Tanjore Y.Raghu, V.Vinayak, Garehatty R.Kanthraj, Banavasi S.Girisha. Study of cutaneous manifestations of diabetes mellitus. *Indian Journal of Dermatology*. 2004;49(2).
- [2] Robbins, Cotran. *Pathologic Basis of Diseases*. 2010. Eight edition.
- [3] She-Fen Chuang, Junne-Ming Sung, Shih-Chen Kuo, Jeng-Jong Huang, Su-Yuan Lee. Oral and dental manifestations in diabetic and nondiabetic uremic patients receiving hemodialysis. *Journal of Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology*. 2005;99:689-95.
- [4] Busato. Im, Ignacio. SA, Brancher. JA, Moyses. ST, Azevedo-Alanis LR. Impact of clinical status and salivary conditions on xerostomia and oral health-related quality of life of adolescents with type 1 diabetes mellitus. *Journal of Community Dental Oral Epidemiology*. 2011; 1600-0528.
- [5] Paul A.Moore, James Guggenheimer, Kenneth R, Etzel, Robert J Weyant, Trevor Orchard. Type 1 diabetes mellitus, xerostomia, and salivary flow rates. *Journal of Oral surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology*. 2001; 92:281-91
- [6] Prabal Pal, Desai NT, Kannan N, Venkatesh Naik Masur, Mariappan Janaraden, Daniel, Bhatt. Estimation of salivary glucose, salivary amylase, salivary total protein and periodontal microflora in diabetes mellitus. *JIDA*. 2003; 74:143-49.
- [7] Sathyapriya S, GO.Bharani, M.Nagalingam, M.Jayanthi, U.Kanagavalli. Potential of salivary protein as a biomarker in prognosis of diabetes mellitus. *Journal of Pharmacy Research*. 2011; 4(7): 2228-29.
- [8] Maria Elena Iopez, Maria Eugenia Colloca, Rafael Gustavo Paez, Judit Nora Schallmach, Myriam Adriana Koss, Amalia Chervonagura. Salivary Characteristics of Diabetic Children. *Brazilian Dental Journal*. 2003 14(1):26-31.
- [9] Scannapieco FA, Torres.G, Levine MJ. Salivary alpha – amylase: role in dental plaque and caries formation. *Critical Reviews in Oral Biology and Medicine*. 1993;4(3/4):301-07.
- [10] Arati S. Panchbhai, Shirish S. Degwekar, Rahul R. Bhowte. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. *Journal of Oral Sciences*. 2010; 52 (3): 359-68.
- [11] Conner.S, Iranpour.B, Mills.J, Rochester. N. Y. Alteration in parotid salivary flow in diabetes mellitus. *Journal of Oral Surgery*. July. 1970; 30(1):
- [12] Straka. M. Oral manifestations of diabetes mellitus and influences of periodontological treatment on diabetes mellitus. *Journal of Citation Reports/ Science Edition*. 2011;112(7):416-20.
- [13] Hanna-Leena Collin, Matti Uusitupa, Leo Niskanen, Anna-Maija Koivisto, Heleena Markkanen, Jukka Meurman. *Journal of Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology*. 1998; 85:680-5.

PARTICULARS OF CONTRIBUTORS:

1. Senior Lecturer, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai, India.
2. Professor and Head, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai, India.
3. Associate Professor, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai, India.
4. Professor, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai, India.
5. Senior Lecturer, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. L. Malathi,
Senior Lecturer, Department of Oral Pathology and Microbiology, Sree Balaji Dental College & Hospital, Bharath University, Chennai-600100, Tamil Nadu, India.
Phone: 9543264959, E-mail: malraj@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 15, 2013**
Date of Peer Review: **Aug 26, 2013**
Date of Acceptance: **Oct 15, 2013**
Date of Publishing: **Nov 10, 2013**