

## “Assessment of Salivary Alpha Amylase level in subjects with Diabetes mellitus -A Cross Sectional Study”

Chethan J<sup>1,\*</sup>, Priyanka BB<sup>2</sup>, Sarfaraz Hassan<sup>3</sup>

<sup>1</sup>Assistant Professor, Dept. of Public Health Dentistry, Subbaiah Institute of Dental Sciences & Research Centre, Shimoga, Karnataka, <sup>2</sup>PG Graduate, Dept. of Conservative & Endodontics, Sharavathi Dental College & Hospital, Shimoga, Karnataka, <sup>3</sup>Assistant Professor, Dept. of Orthodontics, SDM College of Dental Science & Hospital, Dharwad, Karnataka

### \*Corresponding Author:

**Chethan J**

Assistant Professor, Dept. of Public Health Dentistry, Subbaiah Institute of Dental Sciences & Research Centre, Shimoga, Karnataka

Email: chetanjagadish43@gmail.com

---

### Abstract

**Objectives:** The objective of the study was to assess the salivary alpha amylase level in diabetic patient as well as normal individuals using spectrophotometry method.

**Materials and methods:** The present study was conducted among 140 subjects of 35-60yrs of age. The Subjects with diabetes mellitus based on inclusion and exclusion criteria and normal subjects were selected. And the saliva sample was collected from the subjects and salivary alpha amylase level was assessed using spectrophotometry method.

**Results:** The Results of the salivary alpha amylase level in diabetes mellitus group was 2631.73 +77.17  $\mu$ /l and in normal group it was 1634.6+44.17  $\mu$ /l.

**Conclusion:** The results of the present study showed significant increase in the salivary alpha amylase levels in diabetes mellitus group, as compared to normal group. Further studies has to be done with the larger sample size to know the exact range of salivary alpha amylase in normal subjects as well as in diabetes mellitus subjects.

**Keywords:** Salivary alpha amylase, Diabetes mellitus, Spectrophotometer, Normal patients.

---

### Introduction

The prevalence of diabetes mellitus in India was 31.7 million in the year 2000. It is predicted that by the year 2030 diabetes mellitus may raise up to 79.40 million individual in India.<sup>1</sup>

Diabetes mellitus is an iceberg disease. It 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 additional to nephropathy, retinopathy, neuropathy, and cardiovascular diseases.<sup>2</sup>

Diabetes mellitus has been consistently documented to be associated with altered salivary Composition and function. This disrupts the homeostasis of the oral cavity, making it susceptible to various oral ailments. Oral physicians hold the responsibility of recognizing significant associations between certain oral anomalies and diabetes mellitus.<sup>3</sup>

Salivary total protein is a vital component of saliva with salivary proteins, predominantly comprising proline rich proteins, mucin, amylase, immunoglobulins, statherin and antibacterial factors, and these are responsible for most of the functions of saliva.<sup>3</sup>

Saliva is explored to be diagnostic tool as it also meets the demands for an inexpensive, noninvasive and easy to use screening method. The incorporation of salivary diagnostics into clinical practice is gaining

reality and will be of diagnostic value in the prospective future.<sup>4</sup>

The diagnosis of diabetes mellitus has been done routinely in the form of blood glucose level, which is a painful invasive procedure, where in we have to collect the blood from the patient. Patient will not give co-operation in collecting blood which is an invasive procedure.

There by there is a need of an alternative procedure which is non-invasive. Saliva also acts a biomarker for various systemic diseases like diabetes mellitus as it contains various enzymes especially salivary alpha amylase, which is a non-invasive procedure.

Based on this knowledge, in this study we are evaluating salivary alpha amylase levels in saliva of diabetic patient as compared to normal subject, which is a non-invasive method.

### Materials and Methods

**Study design:** The study design was a Cross sectional study. Seventy subjects of age 35-60 yrs newly diagnosed with diabetes mellitus and seventy normal subjects who visited SDM College of Dental Science, Dharwad were recruited for the study.

**Obtaining ethical clearance and permission from the concerned authorities:** The Ethical clearance for the present study was obtained from the Institutional Ethical Committee of SDM College of Dental Sciences & Hospital, Dharwad.

**Sample size determination:** Sample size determination was done based on the pilot study on 10 subjects with 5% error and 95% of confidence interval the estimated sample size was found to be 70 in each group. The subjects who signed the informed consent were enrolled in the study.

**Data collection: Collection of the saliva:** The study Subjects were asked to provide un- stimulated saliva into the salivary tube by Schiffe's method. The saliva samples were immediately taken for sialochemical analysis using spectrophotometer.

**Sialochemical procedure:** The salivary samples collected were transferred into eppendorf tubes and were subjected to centrifugation at 3000 rpm for 15 minutes in order to acquire pure saliva. A starch buffer solution was prepared using 0.02 M sodium phosphate. 0.5ml of centrifuged saliva was added into an eppendorf tube containing 0.5 ml of buffer solution; it was then incubated at 37<sup>0</sup> C so that the reaction starts. Then the solution content was transferred into a test tube and 1 ml of dinitro salicylic acid was added into the test tube which is a stopping agent so that the reaction stops. Then, 10 ml of distilled water was added into the test tube and was kept in the boiling water for 30 seconds. Then the test tube was transferred near spectrophotometer and the wavelength was set at 540 nm and then the solution content into the test tube was added into spectrometer and the reading of the individual sample was noted in microns per litre.

**Statistical analysis**

The data was entered into the computer (MS-Office 2010, Excel sheet data). The data was subjected to statistical analysis using the statistical package (SPSS version 20.0). Independent sample t- test and Chi square test was used to measure the statistical significant differences between the two groups.

**Results**

**Table 1: Distribution of the study subjects in Diabetes mellitus and Normal subjects**

Sl. No.	Study Groups	Diabetes Mellitus group	Mean age	Normal group	Average Age
Group 1	Male	52	45.3	57	41.4
Group 2	Female	18	43	13	44
Total		70	44.15	70	42.7

**Table 1:** Shows the distribution of the study subjects, there were a total of 70 subjects in diabetes mellitus and 70 subjects in the normal group with the mean age of 44.15 in diabetes mellitus and 42.7 in the normal group.

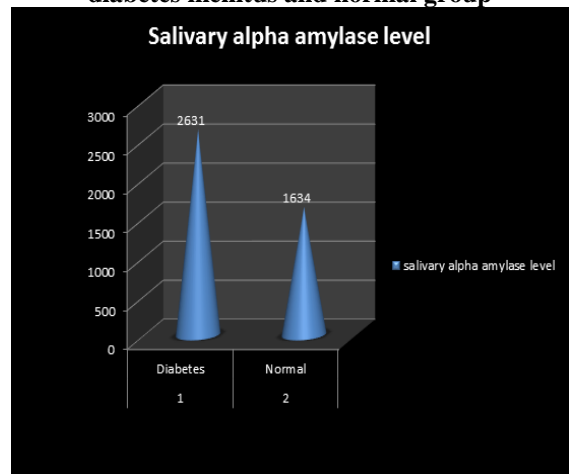
**Table 2: Comparison of salivary alpha amylase in diabetes and normal subjects using Independent sample t –test**

Sl. No.	Groups	Mean	Standard deviation	P value
1.	Diabetes mellitus	2631.73	72.17	0.001*
2.	Normal	1634.60	44.17	

(P ≤ 0.05)

**Table 2:** Shows the comparison of salivary alpha amylase in diabetes and normal subjects, there was statistically significant difference seen in between diabetes mellitus subjects and the normal subjects (P ≤ 0.05).

**Graph 1: Represents mean salivary amylase levels in diabetes mellitus and normal group**



**Discussion**

Salivary alpha amylase acts on long chain polysaccharides i.e. starch and breaks down into monosaccharide glucose.

In the present study there was an increase in the salivary alpha amylase in diabetes mellitus patients as compared to the normal subjects.

In case of diabetes mellitus there will be increase in the glucose in the blood streams and there will be decrease in the glucose in the interstitial tissue, thereby to compensate this glucose regulatory mechanism will get activated in the brain which activates islets of langerhans of the pancreas, acinar cells of pancreas and exocrine gland of salivary gland to produce salivary alpha amylase. There by there will be increase in the salivary alpha amylase in diabetes mellitus patients.<sup>9</sup>

In the present study the total salivary alpha amylase level mean of diabetes mellitus subjects was 2642 µ/l where as in the normal subjects it was 1634 µ/l. which was statistically significant i.e. P=0.001.

The results of the present study is in accordance with the study done by Panchabhai AS et. al, who estimated salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India

and the result showed decrease in the salivary alpha amylase in the controlled diabetes mellitus patients who are under medication as compared to the normal subjects.<sup>3</sup>

Where as in the present study there was an increase in the salivary alpha amylase in freshly diagnosed case of diabetes mellitus that are not under medication. This might be the reason why there was an increase in the salivary alpha amylase in the present study.

The results of the present study is in accordance with the study done by Pal et. al, which showed highly significant positive correlation between salivary alpha amylase and total protein levels in both diabetes mellitus group.<sup>11</sup>

The present study is in accordance with the study done by Malathi et. al, where in Estimation of salivary alpha amylase in diabetic patients as diagnostic tool in early diabetic patients was found the mean score of 2739.48  $\mu$ /l in diabetes patients and normal group was 1740.38  $\mu$ /l.<sup>2</sup>

In the present study newly diagnosed diabetes mellitus patients were subjected for assessing salivary alpha amylase and the salivary alpha amylase level was found more among males as compared to females.

Spectrophotometer was used for assessing salivary alpha amylase which gave definite results of the particular subjects.

### Limitation

The exact normal range of salivary alpha amylase is lacking behind in the present study. Sample size selected was smaller in the present study i.e. 70 in diabetes mellitus group and 70 in the normal group.

### Conclusion

The results of the present study showed significant increase in the salivary alpha amylase levels in diabetes mellitus group, as compared to normal group.

Further studies has to be done with the larger sample size to know the exact range of salivary alpha amylase in normal subjects, as salivary alpha amylase gave promising results in the present study.

### Clinical Implications

Salivary alpha amylase may play a major role in diagnosing diabetes mellitus as it provides a non-invasive procedure and is cost effective as compared to the routine blood glucose estimation which is an invasive procedure.

### References

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *AMJ* 2014;7(1),45-48.
2. Malathi L, Masthan KMK, Balachander N, Babu NA, Rajesh E. Estimation of Salivary Amylase in Diabetic Patients and Saliva as a Diagnostic Tool in Early Diabetic Patients. *Journal of Clinical and Diagnostic Research* 2013;7(11):2634-263.
3. Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. *Journal of Oral Science* 2010;52(3):359-368,
4. Sindhu S, Jagannathan N. Saliva: A Cutting Edge in Diagnostic Procedures; *Journal of Oral Diseases* 2014;1-8.
5. Al-Zahawi SM, Al-Zahawi HA, Al-Qassab ZA. Effects of diabetes mellitus type II on salivary flow rate and some salivary parameters (total protein, glucose and amylase) in Erbil city. *J Bagh College Dentistry* 2012;24(2):2012.
6. Gale EA, Gillespie KM. Diabetes and gender. *Diabetologia*. 2001 Jan;44(1):3-15.
7. Mascarenhas P, Fatela B, Barahona mail I. Effect of Diabetes Mellitus Type 2 on Salivary Glucose – A Systematic Review and Meta-Analysis of Observational Studies. *PLoS One*. 2014;9(7).
8. Longo D, Fauci A, Kasper D, Hauser S .Harrison principles of internal medicine. 2011;18<sup>th</sup> Edition.
9. Scannapieco FA, Torres GI, Levine MJ. Salivary amylase promotes adhesion of oral streptococci to hydroxyapatite. *J Dent Res*. 1995;74(7):1360-6.
10. 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.
11. Pal P, Desai NT, Kannan N, Masur VN, Daniel MJ, Bhatt N (2003) Estimation of salivary glucose, salivary amylase, salivary total protein and periodontal microflora in diabetes mellitus. *J Indian Dent Assoc* 74,143-149.
12. Aydin S (2007) A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. *J Biochem Mol Biol* 40,29-35.
13. Ruterving CO, Reuterving G, Haag E, Ericson T. Salivary flow rate and salivary glucose concentration in patients with diabetes mellitus influence of severity of diabetes. *Diabete Metab*. 1987;13(4):457-62.
14. Belazi MA, Galli-Tsinopoulou A, Drakoulakos D, Fleva A, Papanayiotou PH. Salivary alterations in insulin-dependent diabetes mellitus. *Int J Paediatr Dent*. 1998;8(1):29-33.
15. Amer S, Yousuf M, Siddiqui PQR, Alam J. Salivary glucose concentrations in patients with diabetes mellitus- a minimally invasive technique for monitoring blood glucose levels. *Pakistan Journal of Pharmaceutical Sciences*. 2001;4(1):33-37.
16. Meurman JH, Rantonen P, Pajukoski H, Sulkava R. Salivary albumin and other constituents and their relation to oral and general health in the elderly. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2002;94(4):432-38.
17. Tenovuo J, Lehtonen OP, Vkari J, Larjava H, Vilja P, Tuohimaa P. Immunoglobulins and innate antimicrobial factors in whole saliva of patients with insulin-dependent diabetes mellitus. *Journal of Dental Research*. 1986;65(1):62-66.
18. Collin H-L, Uusitupa M, Niskanen L, Koivisto AM, Markkanen H, Meurman JH. Caries in patients with noninsulin-dependent diabetes mellitus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 1998;85(6):680-85.
19. Hasan HR, Abdulsattar A. Influence of diabetes disease on concentration of total protein, albumin and globulins in saliva and serum: A comparative study. *Iraqi Nat J Chem*. 2015;15(1):1-11.
20. Shirzaii M, Heidari F. Evaluation Chemical Composition of Unstimulated Saliva in patients with Type I Diabetes Mellitus. *Zahedan J Res Med Sci*. 2013;15(1):15-18.

21. Azizi A, Modaberi A. The correlation of blood glucose with salivary glucose level in diabetic patients. *J Islamic Dent Asso.* 2014;25(4):274-77.