

# Salivary alpha-amylase—biomarker for monitoring type II diabetes

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## Abstract

**Background:** Diabetes is one of the most important causes of mortality worldwide. People having diabetes are vulnerable to infectious diseases and have been clinically recognized; which may be because of their deregulated immune system. Hyperglycemia in diabetes is consequence of chronic resistance to insulin and relative insulin deficiency on target cells. Alpha-amylase, a salivary enzyme is shown to increase in diabetic individuals compared to nondiabetics and can be used as a marker for the diagnosis of diabetes.

**Aim and Objective:** The present study was undertaken to investigate the levels of salivary amylase in type II diabetic patients.

**Materials and Methods:** A total of 80 participants in the age range of 30–60 years, were divided into three groups as Group I: Uncontrolled diabetics (30), Group II: Controlled diabetics (30) and Group III: Age- and sex-matched healthy controls (20) were taken for the study. Unstimulated whole saliva was collected for salivary amylase level estimation and blood samples were from the antecubital vein, after 12 h of overnight fasting of each individual for the estimation of blood glucose levels.

**Results:** The mean random blood sugar and glycated hemoglobin A<sub>1c</sub> levels were found to be statistically significant among diabetics and healthy individuals. Salivary amylase levels were much higher in uncontrolled and controlled diabetics compared to healthy individuals showing a significant correlation ( $P = 0.001$ ) between all groups. However, the salivary amylase levels nonsignificant ( $P = 0.060$ ) between the controlled diabetics (Group II) and healthy individuals (Group III).

**Conclusion:** Our study confirms the considerable increase in salivary amylase levels in diabetes patients as compared to healthy individuals. Studies with a larger sample size comprising prediabetics, type I diabetics and type II diabetics in all age groups are required to validate these results. Further, if our results are established, salivary amylase can be used as biomarker for the diagnosis of diabetes and also monitoring it.

**Keywords:** Blood analysis, diabetes, hyperglycemia, salivary amylase

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## INTRODUCTION

Diabetes mellitus is one of the fastest-growing health

challenges of the world in the 21<sup>st</sup> century and has been reported in a staggering 463 million people. India

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ranks second and an estimated 77.0 million people are suffering from diabetes. The number is expected to rise to 134.2 million by the year 2045. Approximately 4.2 million adults aged 20–79 years are estimated to have died as a result of diabetes and its complications in 2019.<sup>[1]</sup>

Blood analysis in the form of Random, Fasting, or Post Prandial blood glucose levels is considered as the only conventional method to diagnose or monitor the disease.<sup>[2]</sup> Hemoglobin A1c (HbA<sub>1c</sub>), the glycosylated Haemoglobin provides an accurate estimate of glucose levels in the blood over the past 23 months and helps overcome the inconsistency in records of at-home monitoring.<sup>[3]</sup> However, monitoring of blood glucose levels at frequent intervals can cause discomfort and mental trauma to many individuals. Hence, the use of saliva through its easy and noninvasive collection procedure has made it valuable in young, old and infants.

Saliva is also becoming a popular biofluid for point of care technology and salivary diagnostics is being integrated for disease diagnosis, clinical monitoring and making significant clinical decisions.<sup>[4]</sup>

One such metabolite, the alpha-amylase, a salivary enzyme belonging to the glycoside hydrolase family is produced by salivary glands, mainly parotid. It functions to digest macromolecules such as carbohydrates and starch. A significant increase in its levels in diabetic individuals as compared to the nondiabetics resulting in excessive production of glucose makes it a marker for the diagnosis of diabetes. Few studies have also indicated that the use of salivary alpha-amylase inhibitor with reduced absorption of complex carbohydrates could be effective in controlling blood sugar.<sup>[5]</sup>

Thus there is a need for such more studies supporting the previous studies. Hence, the present study was undertaken to explore the use of salivary amylase estimation as a biomarker for diagnosing and monitoring the glucose levels in type II diabetic patients.

## MATERIALS AND METHODS

In the present study, a total of 80 participants in the age range of 30–60 years, were divided into three groups, namely Group I: Uncontrolled diabetics, Group II: Controlled diabetics and Group III: Age- and sex-matched healthy controls having 30, 30 and 20 participants, respectively. An Institutional Ethics Committee approval and a written informed consent were obtained from each participant. Individuals with severe diabetic complications,

other systemic illnesses, or on medication for any other diseases that could affect salivary amylase levels were excluded from the study.

The following are the inclusion and exclusion criteria which are considered in the study.

### Inclusion criteria

- Patients who are attending the outpatient clinics of Dhiraj General Hospital, a constituent of Sumandeep Vidyapeeth, Piparia, Vadodara
- Patients aged between 30 and 60 years of age
- Diabetic patients who have elevated blood glucose levels (Group I), diabetic patients who have regulated blood glucose levels (Group II), normal healthy individuals (Group III) are included.

### Exclusion criteria

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

Unstimulated whole saliva was collected from each participant by asking them to gently drool for 1 min in a sterile container. Saliva samples were collected between 9 and 11 am to avoid diurnal variation and stored at 4° C till analysis. Each sample of 5 ml was centrifuged at speed of 5000 rpm for 5 min to remove any debris thereof. Thereafter, salivary amylase levels of each sample were determined using semiautomatic analyzer with direct substrate method (kinetic enzyme assay). The random blood sugar (RBS) level of each participant was also estimated using a glucometer. Antecubital venous blood samples had been taken after 12 h of overnight fasting of each individual. The collected samples were used for the estimation of fasting blood glucose and HbA<sub>1c</sub> levels.

## RESULTS

The mean age of healthy individuals in the present study was 46.35 years, among the controlled diabetics it was 54.27 years and that among uncontrolled diabetics it was 50.83 years. A statistically significant correlation was observed in the age among the three groups. The mean RBS level in the healthy group was 133 mg/dl, whereas subsequently higher levels were observed, in controlled diabetics 164 mg/dl and uncontrolled diabetics. The mean RBS levels were found to be 204 mg/dl, showing a statistically significant correlation among the three groups ( $P = 0.02$ ) [Table 1].

Glycated HbA<sub>1c</sub> level was found to be well controlled in healthy individuals (HbA<sub>1c</sub> = 5.31%), slightly higher

in controlled diabetics ( $HbA_{1c} = 6.49\%$ ) and much higher levels of glycated  $HbA_{1c}$  were observed in uncontrolled diabetics (mean = 8.39%) depicting a poor glycemic control. These findings were statistically significant ( $P = 0.001$ ) [Table 2].

Salivary amylase levels were much higher in uncontrolled (mean = 114.7 u/ml) and controlled (mean = 94.7 u/ml) diabetics compared to healthy individuals (mean = 85.7u/ml) showing a significant correlation ( $P = 0.001$ ) between all these three study groups [Table 3]. Interestingly, while comparing salivary amylase levels between the controlled diabetics (Group II) and healthy individuals (Group III), the mean difference of this parameter was found to be nonsignificant ( $P = 0.060$ ) [Table 4].

## DISCUSSION

Diabetes is a metabolic disorder characterized by abnormalities in the metabolism of carbohydrate, lipid and protein that results either from a profound or absolute insufficiency of insulin secretion (Type 1) and/or target tissue resistance to its cellular metabolic actions (Type 2).<sup>[6]</sup>

Type II diabetes is reported in about 90%–95% of individuals living with diabetes. It is due to a chronic resistance to insulin action in target cells and further relative insulin deficiency leading to hyperglycemia. It is associated with various complications and mainly affects the heart, kidneys, eyes and nerves. It also causes impairment of salivary gland function and thus altering the oral cavity homeostasis. This leads to a decrease in the pH of saliva and xerostomia leading to complications such as caries, gingivitis and periodontitis.

Individuals with any type of diabetes are at an increased risk for developing serious health complications that can affect the heart, blood vessels, eyes, kidneys and nerves. They are also at a higher risk of developing infections, which significantly shortens life expectancy.<sup>[4]</sup> Furthermore, there exists a considerable heterogeneity within the diabetic population with regard to the development and progression of such complications.<sup>[7]</sup>

Besides these multi-organ damages, it also impairs salivary functions significantly resulting in qualitative and quantitative changes in salivary composition and flow. This deteriorates the homeostasis of the oral cavity, making it vulnerable to various oral ailments. Studies on various salivary metabolites have been being carried for the diagnosis and prognosis of diabetes.

**Table 1: Inter-group relationship of RBS and various study groups**

Factor	Group	Number	Mean (mg/dl)	SD	P
RBS	Uncontrolled diabetics	30	204.1	28.9	0.02*
	Controlled diabetics	30	164.4	18.8	
	Healthy individuals	20	133.77	9.46	

\*Statistically significant

**Table 2: Intergroup relationship between  $HbA_{1c}$  and group**

Factor	Group	Number	Mean (%)	SD	P
$HbA_{1c}$	Uncontrolled diabetics	30	8.39	0.84	0.001*
	Controlled diabetics	30	6.49	0.60	
	Healthy individuals	20	5.31	0.65	

\*Statistically significant

**Table 3: Intergroup relationship between salivary amylase and study group**

Factor	Group	Number	Mean (u/ml)	SD	P
Salivary Amylase	Uncontrolled diabetics	30	114.7	11.66	0.001*
	Controlled diabetics	30	94.7	5.11	
	Healthy individuals	20	85.7	16.21	

\*Statistically significant

**Table 4: Intergroup comparisons of various parameters among the three groups**

Dependent Variable	Group	???	Significance
Salivary Amylase	I	2	0
		3	0
	II	1	0
		3	0.06
	III	1	0
		2	0.06
RBS	I	2	0
		3	0
	II	1	0
		3	0
	III	1	0
		2	0
$HbA_{1c}$	I	2	0
		3	0
	II	1	0
		3	0
	III	1	0
		2	0

Moreover, persistent hyperglycemia alters blood vessels and basement membrane permeability of salivary glands, leading to an increase in glucose percolation as well as other small molecules from the blood to saliva through gingival crevices.<sup>[4]</sup> Estimation of these metabolites entering saliva or produced by salivary glands, using simple biochemical tests can be useful for both early detection and monitoring of the disease.

In the present study, majority of diabetic patients were male ( $n = 33$ ) and in the sixth decade of life. A statistically significant correlation was found ( $P = 0.032$ ).

Mean RBS levels were found to be significantly increasing from healthy individuals (133 mg/dL) to controlled diabetics (164 mg/dL) to uncontrolled diabetics (204.5 mg/dL). The *P* value was found to be significant (*P* = 0.02).

A very high statistically significant increase (*P* = 0.001) was found in salivary amylase levels in uncontrolled diabetics (114.7 u/dl) as compared to controlled (94.7 u/dl) and healthy individuals (85.7 u/dl). This could be hypothesized by the fact that reactive oxygen species (ROS) are generated in diabetes mellitus. The ROS result in activation of nuclear factor Kappa B (NF- $\kappa$ B) and induction of inflammatory cytokines gene expression in salivary acinar cells from these cells.<sup>[8]</sup> A positive correlation was found between HbA<sub>1c</sub> and salivary amylase levels in diabetics. A significant increase in salivary amylase levels among diabetic patients in our study group was consistent with the findings of previous studies done by Pal *et al.*<sup>[9]</sup> Moreover, there was a significant increase in salivary amylase levels in diabetic patients than in the healthy individuals in the study conducted by Malathi *et al.*<sup>[10]</sup> Sathyapriya *et al.* in their research done on the potential of salivary protein as a biomarker in the prognosis of diabetes mellitus found that total salivary protein levels were higher in the uncontrolled and controlled diabetic groups than in the healthy nondiabetic group and that the differences were highly significant.<sup>[11]</sup>

Intergroup comparison of all the parameters showed a statistically significant correlation between RBS and HbA<sub>1c</sub> levels (*P* = 0.00) in diabetics and healthy individuals. But a nonsignificant correlation (*P* = 0.060) of these parameters was found between controlled and uncontrolled diabetics.

In the present pandemic of COVID-19 infection, several reports have indicated that older patients with chronic diseases, including diabetes, are at a higher risk for severe COVID-19 and mortality.<sup>[12-14]</sup> Evidence shows that hyperglycemia worsens the prognosis and increases the risk to death.<sup>[8,15]</sup>

Several studies have confirmed that salivary amylase causes increased accumulation of plaque in the interdental areas.<sup>[10]</sup> Increased accumulation of plaque in individuals leads to increased concentration of salivary glucose in the oral cavity, which favors the proliferation of both aerobic and anaerobic bacteria in the gingival plaque leading to diabetes.

Salivary amylase also plays a role in the etiology of dental caries and as caries activity is high in diabetics,<sup>[16,17]</sup> studies

considering this triad can be undertaken needed to substantiate the role of salivary amylase in increasing the cariogenic activity in diabetes patients.

## CONCLUSION

The present study confirms the significant increase in salivary amylase levels in diabetes mellitus patients as compared to healthy individuals. Salivary amylase can be used as biomarker for diagnosis of diabetes and also monitoring it. It also opens up avenues for early diagnosis in prediabetics who are at a higher risk for Type II diabetes. Studies with a larger sample size in prediabetics, Type I diabetics and Type II diabetics are needed to substantiate these results.

Salivary amylase is said to play a role in the etiology of dental caries and caries activity is also reported to increase in diabetics, hence studies considering this triad are also needed to substantiate the role of salivary amylase in increasing the cariogenic activity in diabetes patients.

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## Conflicts of interest

There are no conflicts of interest.

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