Estimation of Serum Glucose and Salivary Glucose in Type II Diabetes Mellitus

Reena C.S[1], A.P. Indira[2], Maria Priscilla David[3]

ABSTRACT

Introduction: Diabetes Mellitus is a complex multisystem disorder Characterized by relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. Type II Diabetes Mellitus (T2DM) accounts for 90% to 95% of all diabetic patients. Diabetes is known to influence changes in salivary composition and function. There is altered expression of alpha amylase and cyclic AMP receptors. The change in secretory proteins along with increased basement membrane permeability leads to increased expression of alpha amylase into their secretions, thus raising their levels in saliva. Study objective to assess if salivary alpha amylase can serve as a potential diagnostic indicator of type 2 Diabetes Mellitus

Material and methods: A total of 40 subjects (20 T2DM patients and 20 controls) selected in the age range of 30 – 65years. inclusive of both the genders. Fasting unstimulated saliva samples were collected from the study and control groups. The obtained values were subjected to statistical analysis using student's t test.

Results: The mean fasting salivary alpha amylase level was found to be higher in Type II DM (11.62 u/l) when compared with controls (2.5u/l). The observed difference was found to be statistically significant, (P<0.001). The cut off value of fasting salivary alpha amylase observed in this study was ≥5 u/l. The observed sensitivity was 95% and specificity was 100%.

Conclusion: This study suggests that saliva can be used as a diagnostic media for assessing salivary alpha amylase as a potential diagnostic indicator of Type II diabetes mellitus.

Keywords: Diabetes Mellitus; Saliva; Salivary Alpha Amylase

INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder characterized by abnormalities in the metabolism of carbohydrate, lipid and protein that result either from a profound or an absolute insufficiency of insulin secretion (Type 1 DM) and/or target tissue resistance to its cellular metabolic actions.[1] (Type 2 DM). DM accounts for the second highest disease in the world. The prevalence of DM in India ranges from 5–17%.[2] T2DM is more common which estimates, up to 90 to 95% of all diabetic patients.[3] As per Indian Diabetic Federation, there are currently 61.2 million diabetic individuals in India of which T2DM16, which constitutes more than 95% of diabetic populations.[4] Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels leading to increased levels of complications.[5]

Diabetes most often remains undiagnosed as it may present with no symptoms. Without timely diagnosis, morbidity and mortality rate from diabetes rise exponentially.[6] Hence, early diagnosis is necessary which would play a significant role in successful clinical treatment.[7] Monitoring of individuals with DM, requires frequent drawing of blood which is often cumbersome to the patient. Thus, there is a splurge of interest in non-invasive diagnostic method which entails the need for other body fluids.[8] In this regard, saliva is an organic bio-fluid and a medium for monitoring the health and diseased state of an individual.[9] It expresses the local and systemic alterations in disease. Saliva, as a diagnostic medium can be considered due to the fact that, the substances present in the serum are also seen in saliva.[6]

Alpha-amylase, present in saliva is one of the principal salivary proteins. It is a digestive metalloenzyme synthesized in the acinar cells of salivary gland and stored in secretory granules which accounts for up to 50% of total salivary proteins.[10] DM has been consistently documented to be associated with altered salivary gland function, salivary composition i.e. proteins and salivary enzymes.[11] There is altered expression of alpha amylase and cyclic AMP receptors. This change in secretory proteins along with increased basement membrane permeability leads to increased expression of alpha amylase into their secretions and to the oral cavity.[1]

With this background, this study was taken up to estimate salivary alpha amylase and to asses if salivary alpha amylase can serve as a potential diagnostic indicator of type II diabetes mellitus.

Study objectives were to estimate salivary alpha amylase levels in healthy controls and in type II diabetes mellitus, to compare salivary alpha amylase levels between healthy controls and type II diabetes mellitus and to assess if salivary alpha amylase can serve as a potential diagnostic indicator of Type II diabetes mellitus

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MATERIAL AND METHODS

A total of 40 subjects were selected in the age range of 30 – 65 years inclusive of both the genders. Detailed case history and clinical examination was carried out. Fasting blood and saliva were used as study samples. The salivary samples were collected from both controls and the study subjects to estimate salivary alpha amylase. Fasting blood glucose was advised for controls & HbA1c values were advised for the study subjects to confirm diabetes mellitus.

Sampling of Blood: In controls, 2ml of fasting blood sample was drawn from the antecubital vein following aseptic procedure and blood samples were labeled. The sample was centrifuged at 3000rpm for 15 minutes for separation of serum, which was then transferred to a plastic fluoride oxalate tube to estimate the serum glucose level.

In study subjects, 2ml of fasting blood sample was drawn from the antecubital vein following aseptic procedure. This was transferred to an EDTA tube for estimation of HbA1c.

Sampling of Saliva: Subjects were asked to rinse the mouth thoroughly with water. The salivary samples were collected in sterile containers by instructing them to allow saliva to collect naturally in mouth and spit it, into the container. Around 2ml of saliva was collected and stored in the ice pack container. The collected samples were transported to the lab where it was centrifuged at 4000rpm for 15 minutes to clear the supernatant of saliva.The salivary alpha amylase level was then estimated by enzymatic colorimetric assay.

Inclusion criteria

Healthy individuals with normal level of blood glucose. Patients diagnosed with Type II Diabetes Mellitus (including those undergoing treatment).

Exclusion criteria

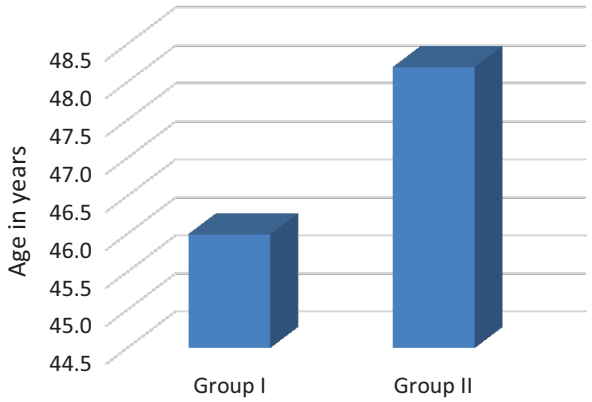
Pregnancy patients with salivary gland disorders. Patients on treatment for salivary gland diseases. Patients who had undergone surgery of the salivary glands. Patients who have undergone radiotherapy / chemotherapy for head and neck malignancy. Type II Diabetic patients with any other systemic diseases/habits.

STATISTICAL ANALYSIS

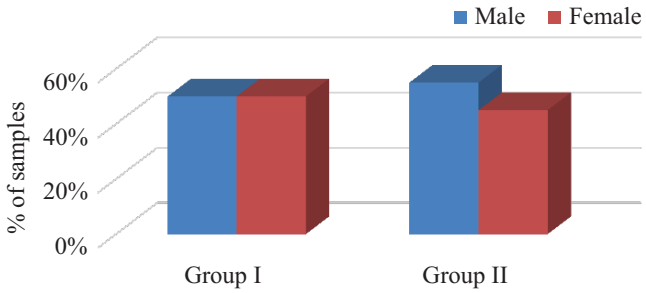
The obtained values of salivary alpha amylase were subjected to statistical analysis using student's t test and Receiver Operating Characteristic (ROC) curve was established.

RESULTS

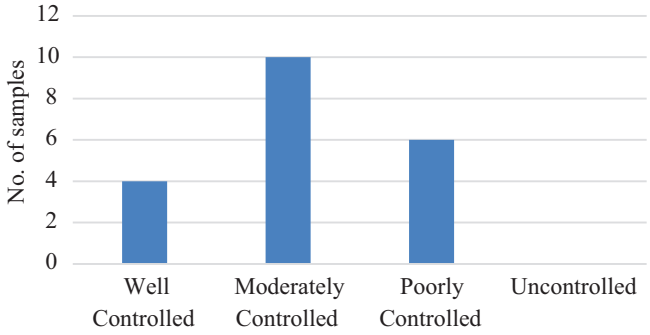
A total of 40 subjects were selected in the age range of 30 – 65 years inclusive of both the genders. The Mean age in group I was 46 years and in group II was 48.20 years. In group II 45% were females and 55% males. There were more number of patients in moderately controlled, followed by poorly controlled, least in well controlled and none in uncontrolled group. In group I, the mean salivary alpha amylase was 2.5 u/land In group II, it was observed as 11.62 u/l. Higher mean salivary alpha amylase was recorded in group II compared to control group. The observed difference between the groups was found to be statistically significant (P<0.001). The ROC Curve cut off value of salivary alpha amylase observed was ≥5 u/l. The sensitivity was 95% and specificity was 100%.



Graph-1: Mean age distribution in total sample



Graph-2: Gender distribution in total sample



Graph-3: Distribution of subjects in group ii according to HbA1c

Group	Age range (years)	Mean age (years)
Group I	30-61	46.00
Group II	32-65	48.20
Mean age in group I was 46.00 years and in group II was 48.20 years.		

Table-1: Mean age distribution in total sample

Group	Control Group		Study Group	
	n	%	n	%
Female	10	50%	9	45%
Male	10	50%	11	55%
Total	20	100%	20	100%
In group I 50% were females and 50% males In group II 45% were females and 55% males				

Table-2: Gender distribution in total sample

DISCUSSION

Chronic hyperglycemia is associated with microvascular and macrovascular complications, which decreases quality of life and life expectancy.[12] Hence early diagnosis plays a potent role in rendering effective treatment to avoid future complications.[13] Saliva, being a biological medium is considered as the 'mirror of the body', and a perfect medium to be explored for assessing biological markers in health and diseases.[14] In diabetic patients, the salivary gland manifests an altered expression of alpha amylase and cyclic AMP receptors.[1] The alpha-amylase is a digestive enzyme produced by salivary glands that hydrolyze starch into dextrins and monosaccharides. These monosaccharides are composed of glucose units, and an increase in these glucose units leads to hyperglycemia.[15] In diabetic patients, the salivary gland manifests an altered expression of alpha amylase and cyclic AMP receptors. This establishes changes in the secretory proteins and also increased basement membrane permeability. This leads to an increased expression of alpha amylase into the salivary secretions and to the oral cavity.[1]

Mean age distribution in group I was 46 years and that of group II was 48.20 years. (Table 1, Graph 1) This is in accordance with the study conducted by Malathi et al.[16] and Chethan J et al[11] where similar mean age distribution was observed. Most often, the mean age for disposition to diabetes mellitus occurs during 4th decade of life.

The distribution of male subjects was more than females. This is in accordance with the study conducted by Aregbesola et al.[17] where the author observed more number of male subjects. In group II, more number of subjects were observed in moderately controlled (n=10) followed by poorly controlled (n=6), less in well controlled (n=4) and nil in uncontrolled group. (n=0) (Table 3, Graph 3) This may be due to the fact that patients would have been on medication for the treatment of the disease along with good co-operation towards diet and medication.

Group II	HbA1c values	No. of patients
Well Controlled	≤8%	4
Moderately Controlled	>8 - ≤10%	10
Poorly Controlled	>10 - ≤12%	6
Uncontrolled	>12%	0
There were most number of patients in moderately controlled, followed by poorly controlled, least in well controlled and nil in uncontrolled group.		

Table-3: Distribution of subjects in group ii according to HbA1c

	Observed Range (u/l)	Mean value (u/l)
Group I		
Salivary alpha amylase	1-4	2.5u/l
Group II		
Salivary alpha amylase	4-19	11.62
In group I the mean salivary aipha Amylase was 2.5u/l and In group II it was observed as 11.62 u/l		

Table-4: Estimation of salivary alpha amylase in group I and group II.

Estimation of mean salivary alpha amylase in group I

In group I, the observed mean value of fasting salivary alpha amylase was 2.5u/l.

The observed values were within the normal limit (n = ≤ 5 u/l). Hence, were grouped as controls (Table 4).

In group II, the observed value of mean fasting salivary alpha amylase was 11.62u/l there was marked increase in salivary alpha amylase in the study subjects. The observed values were found to be above the normal limit. (n = ≤ 5 u/l) (Table 4).

This is in accordance with Mortazavi et al[1], Sathya priya et al[18], Maleki et al,[19] Chethan J et al[11] where they also observed increased mean fasting salivary alpha amylase value.

In case of diabetes mellitus there will be increase in glucose in the blood streams and there will be decrease glucose in the interstitial tissue. In general 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 acinar cells of salivary gland.[15] Added to this mechanism, the salivary gland of diabetic patients manifests an altered expression of alpha amylase and cyclic AMP receptors which leads to changes in secretory proteins and increased basement membrane permeability, which leads to increased expression of alpha amylase into their secretions and to the oral cavity.[1]

Comparison of salivary alpha amylase in group I and II

The mean fasting salivary alpha amylase in group I was 2.5u/l and in group II was 11.6u/l. There was great increase

Group	n	Mean	Std Dev	SE of Mean	Mean Difference	t	P-Value
Group I	20	2.05	1.20	0.27	-9.572	-10.415	<0.001*
Group II	20	11.62	3.93	0.88			
*denotes significant difference. Higher mean salivary alpha amylase was recorded in group II (Diabetes Mellitus) compared to control group. The difference in mean salivary alpha amylase between the groups found to be statistically significant (P<0.001)							

Table-5: Comparison of salivary alpha amylase between Group I and II

Area Under the Curve	Std Error	P-Value	95% Confidence Interval	
			Lower Bound	Upper Bound
0.9975	0.0042	<0.001*	0.989	1.000

Table-6: Receiver operating characteristic curve

in the mean salivary alpha amylase in group II when compared with group I. Salivary alpha amylase levels were significantly higher in diabetic subjects than in controls.

This indicates that, the salivary alpha amylase values increase in diabetes mellitus.

The observed difference was found to be statistically significant (P<0.001). (Table 5)

This is in accordance with the study conducted by Mortazavi et al1, Sathya priya et al[20], Malathi et al[16], Chethan J et al[11], Aydin et al.[20] Where they also observed salivary alpha amylase levels to be higher in diabetes than in controls.

In contrast to this study, Indira et al[21] and Panchbhai et al[22] found salivary alpha amylase levels to be lower in type II diabetes when compared with controls.

The changes in basement membrane permeability may also lead to an enhanced leakage of serum derived components into saliva via gingival crevices1. The annular receptor expression levels of amylase and cyclic AMP receptors in diabetic patients will increase, as the basement membrane permeability of salivary glands is increased. This leads to the penetration of salivary proteins such as amylase into their secretions and to the oral cavity. This supports for increase in salivary alpha amylase among diabetes mellitus.[19]

In this study, ROC curve established the cut off value of salivary alpha amylase to be ≥5 u/l in type II DM. (Table 6, Graph 5) ROC curve demonstrated salivary alpha amylase sensitivity of 95% and specificity of 100%. In this study the subjects with values of ≥5 u/l can be considered as subjects confirmed with diabetes mellitus.

In outlook of this study, we came across certain limitations. Saliva does not reflect the exact concentration of contents of blood; highly viscous saliva cannot be utilized in the study.

This study recommends the exploration of saliva as a diagnostic medium. The contribution of this study, is that it can provide early detection of diabetes mellitus, which would help in preventing oral mucosal disorders and future complications. Saliva as a biological and diagnostic tool, meets the demand of being inexpensive, painless, easy to collect, non-invasive and with minimal training. We endorse the use of salivary alpha amylase as a potential diagnostic indicator of type II diabetes mellitus.

CONCLUSION

Saliva has been in the spotlight of the researcher's attention due to its possession of different enzymes and molecules. More over the substances present in the serum is also reflected in saliva. Saliva offers distinctive advantages as it is collected non-invasively by persons with modest training, and it offers a cost-effective approach, permitting multiple samples. Estimation of salivary alpha amylase can be used as a potential diagnostic aid to diagnose type II diabetes mellitus. This may be materialized in screening of large samples at community level, health care centres and for epidemiological studies.In culmination, we exhort the use of saliva as a medium for various diagnostic procedures in routine clinical practice. Further scrutiny with enormously meticulous and standard techniques should be used as a template in the diagnosis of type II diabetes mellitus.

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