Assessment and Comparison of Total Salivary Protein and Salivary Flow Rate among Type I, Type II Diabetics and Healthy Controls

B.Niveditha[1], M. Kavitha[2], Mutum Sangeeta Devi[1], J. Manju[3], C.K. Vishnu Priya[4], D.K.S.Lakshminrusimhan[5]

[1]Senior Lecturer, Madha Dental College & Hospital Chennai, [2]Reader, Madha Dental College & Hospital, Chennai, [3]Senior Lecturer, Thai Moogambigai Dental College & Hopsital, Dr MGR Educational and Research Institute ,Chennai, [4]Senior Lecturer, Ragas Dental College & Hospital, Chennai, [5]Senior Lecturer, R.V.S. Dental College & Hospital, Coimbatore

Abstract

Introduction: Diabetes mellitus characterized by either absolute insulin deficiency (Type I) or target tissue resistance (Type II) is associated with oral complications like xerostomia, gingivitis, periodontitis, odontogenic abcesses and soft tissue lesions of the oral mucosa. Saliva is a unique biological fluid and is also a characteristic biomarkers for different diseases.Several classes of drugs are found to be associated with dry mouth or salivary gland dysfunction or hypofunction, which in turn influences concentration of salivary proteins. This leads to changes of oral health status among individuals using these drugs. **Aim of the Study:** To evaluate and compare the total salivary protein and salivary flow rate among Type I Diabetics, Type II Diabetics and healthy controls. **Materials and Methods:** A total of 60 individuals have participated in the study which include 20 with Type I diabetes, 20 with Type II diabetes and 20 healthy controls. The study was prospective in nature. Patients were asked not to eat or drink 2 hours before the time of saliva collection. The samples were collected in the same time of the day to avoid circadian variations. unstimulated saliva was collected using spit technique. Patient was instructed to spit the saliva in graduated containers for a period of two minutes. The flow rates were determined visually from graduated salivary containers as ml/min. After measuring the saliva volume the saliva sample was stored in deep freeze until protein estimation. The total salivary protein in each salivary sample was determined using BioRad Protein Assay Dye Concentrate method using BSA standard. **Results:** On comparing the total salivary protein among Type I, Type II Diabetics and healthy controls, a significant difference in total salivary protein was found among Type I Diabetics and healthy controls and also among Type I and Type II Diabetics and there was a insignificant difference in Type II Diabetics and controls. There was an insignificant difference in total salivary flow rate among Type I and Type II Diabetics and healthy controls. **Conclusion:** A significant difference in total salivary protein level among the diabetic and non diabetics emphasized that protein utilization by other biochemical metabolic pathways has an overall systemic response to glucose intolerance .With regards to salivary flow rate ,the inconsistent results obtained may be due to the duration of diabetes, age range of patients and metabolic control of patients, class of drugs taken by the patient .

Keywords: *Diabetes mellitus, Salivary flow rate, Protein, Glucose*

Corresponding Author:
Dr. B. Niveditha
Senior Lecturer, Madha Dental College & Hospital
Chennai
E-mail: bniveditha14980@gmail.com

Introduction

Diabetes mellitus is a syndrome characterized by abnormalities in carbohydrate, protein and fat metabolism that result either from a profound or absolute deficiency

of insulin(Type I), or from target tissue resistance to it's cellular metabolic effects(Type II).[1]It is found to have a multitude of oral manifestations in the oral cavity.[2]

Saliva is a unique biological fluid, which when compared to other diagnostic media ,such as tissue samples, serum, CSF),saliva sample is easily collectable, cost effective, non invasive diagnostic tool for research and is often preferred as an alternative diagnostic approach.[3]

Alterations in salivary parameters causes disorders of the hard and soft tissues of the mouth leading to gingival lesions, increased prevalence of caries and finally bad oral health. Dry mouth is a complaint among diabetic patients and may be associated with several classes of drugs , which in turn influence concentration of salivary proteins. This leads to changes of oral health status among individuals using these drugs.[4]

Since saliva is a non diagnostic tool the present study aims to detect and compare the difference in salivary flow rate and total salivary protein among Type I and Type II diabetics and healthy controls, which may be useful in describing and further understanding of oral findings in this condition.

Methodology

The present study is a prospective study which includes a total sample of 60 patients which includes 20 in the Type I diabetic group, 20 in the Type II diabetic group and 20 in the healthy control groups. Patients with symptoms of diabetes including polyphagia, polydipsia and polyuria, with a non fasting glucose level of greater than 200 mg/dl and HbA1c level greater than 6.5% were included in the study. The control group consisted of gender matched non diabetic subjects without symptoms of diabetes. Patients with other systemic illness, smoking/alcohol habits, those treated on radiotherapy and pregnancy were excluded from the study.

Patients were asked not to eat or drink 2 hours before the time of saliva collection. The samples were collected in the same time of the day to avoid circadian variations. Unstimulated saliva was collected using spit technique. Patients were instructed to spit the saliva in graduated containers for a period of two minutes. The flow rates were determined visually from graduated salivary containers as ml/min. After measuring the saliva volume the saliva sample was stored in deep freeze until protein estimation. The total salivary protein in each sample was determined using BioRad Protein Assay Dye Concentrate method using BSA standard. Bio-Rad Protein Assay Dye Reagent Concentrate is a colormetric assay for protein concentration similar to the Lowry assay, but with the following improvements: Within 15 minutes, the reaction reaches it's maximum colour change and the change in colour is not more than 5% in one hour. A standard curve is prepared each time the assay is performed .

BioRad assay is prepared by diluting the assay dye in the ratio of 1:5, where 2 ml of Bio Rad dye is mixed with 10 ml of distilled water and the mix is stirred well. The volume of the bovine serum albumin sample used as standard is 5µl and the sample is also 5 µl. 100 µl of diluted dye is then treated with 5µl of the standard and 5µl of the sample and then ultraviolet spectrometer with a wavelength of 595nm is then used to determine the total protein in each sample of saliva.

Statistical Analysis

Turkey HSD test was used to correlate the difference in salivary proteins and salivary flow rate amomg diabetic and healthy controls.The mean difference is taken as significant at the .05 level. The SPSS 13 software analysis is used.

Results

A comparison of total salivary protein among Type I, Type II Diabetics and healthy controls were performed in male and female subjects . The total salivary flow rate was also compared among TypeI, TypeII Diabetics and healthy controls with the following results:

On comparing the total salivary protein among the Type I, Type II diabetics and healthy controls in male subjects, it was found that the mean difference between the total salivary protein in controls and type

I diabetics was -1.96046 with a P value of **0.015** which was statistically significant. The mean difference between the total salivary protein in controls and type II diabetics was -.13536 with a P value of **0.977** which was statistically insignificant. The mean difference between the total salivary protein in type I and type II diabetics was 1.82510 with a P value of **0.025** which was statistically significant.

On comparing the total salivary protein among Type I, Type II diabetics and healthy controls in female subjects, it was found that the mean difference between the total salivary protein in controls and type I diabetics was -2.78661 with a P value of **0.006** which was statistically significant. The mean difference between the total salivary protein in controls and type II diabetics was -1.05711 with a P value of **0.418** which was statistically insignificant. The mean difference between the total salivary protein in Type I and Type II diabetics was 1.72950 with a P value of **0.110** which was statistically insignificant.

On comparing the salivary flow rate of controls with type I and type II diabetics, it was found that the mean difference in salivary flow rate between controls and type I diabetics was 0.2245 with a P value of **0.089** which was statistically insignificant. The mean difference in salivary flow rate between controls and type II diabetics was 0.0790 with a P value of **0.731** which was statistically insignificant.

On comparing the salivary flow rate of type I diabetics with controls and type II diabetics, it was found that the mean difference of salivary flow rate between type I diabetics and controls was -.2245 with a P value of **0.089** which was statistically insignificant. The mean difference in salivary flow rate between type I diabetics and type II diabetics was -.1455 with a P value of **0.351** which was statistically insignificant.

On comparing the salivary flow rate of type II diabetics with controls and type I diabetics, it was found that the mean difference of salivary flow rate between type II diabetic and controls was -.0790 with a P value of **0.731** which was statistically insignificant. The mean difference of salivary flow rate between type II diabetics and type I diabetics was 0.1455 with a P value of **0.351** which was statistically insignificant.

Discussion

Saliva is a unique biological fluid, with an important role in oral physiology and highly sensitive methods has succeeded to reveal the acceptable sensitivity and specificity of salivary biomarkers in term of different local and systemic conditions.[3]. Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and this resulst in raised percolation of components such as glucose, amylase and protein from blood, thus raising their levels in saliva. If the glycation of salivary proteins is linked with glycated proteins in blood and blood glucose, it can be used to detect diabetes at an early stage.[5] Decreases in salivary flow rate or oral dryness occurring in diabetes can be multifactorial.[6]

In the present study, we have aimed to compare the total salivary protein and salivary flow rate among the type I diabetics, Type II diabetics and healthy controls, in order to aid in reaching firm conclusions about their alterations in diabetics as compared to healthy non-diabetics and to assess any significant correlations that may exist among the various parameters under consideration in the present study. The mean difference in total salivary protein in controls and type I diabetics was -1.96046 with a P value of 0.015 which was statistically significant. The mean difference between the total salivary protein in controls and type II diabetics was -.13536 with a P value of 0.977 which was statistically insignificant. The mean difference between the total salivary protein in type I and type II diabetics was 1.82510 with a P value of 0.025 which was statistically significant.

The results of our study are in accordance to the study conducted by **Laisi T.J et al (2012)[7] and SyedShahbaz et al (2017)[8].**

In the study conducted by **Laisi T.J et al (2012)[7]** in a sample of total of 40 subjects of 20 type II diabetics

and 20 healthy controls, it was found that the P value was greater than 0.005 which was statistically insignificant. In the study conducted by **Syed shahbaz et al (2017)[8]**, in a sample of total of 100 patients of 50 type 1 diabetics and 50 healthy controls, it was found that the P value was <0.01 which was statistically significant.

The results of our study are in contradiction to the results of the study done by **Prathibha et al (2013)[9], Indira M et al (2015)[10], Juan Aitken ,Saveendra et al (2015)[11] .**

In the study conducted by **Prathibha et al (2013)[9],** in a total sample of 60 subjects, 30 in the diabetic group and 30 as healthy controls,it was found that the P value was 0.000 which was statistically highly significant. In the study conducted by **Indira et al (2015)[10],** in a total sample of 40 subjects, 20 in the type 2 diabetic group and 20 in the healthy controls, it was found that the P value was <0.001 which was statistically significant. In the study carried out by **Juan Aitken Saveendra et al (2015)[11],** in a total sample of 74 patients, it was found that the P value was 0.000 which was statistically highly significant.

The contradiction could be explained by the fact that there is a variation in the salivary fluid secretion among the diabetic patients and varying oral health conditions like periodontitis influence the leakage of proteins from the gingival crevicular fluid.

On comparing the salivary flow rate of controls with type I and type II diabetics, it was found that the mean difference in salivary flow rate between controls and type I diabetics was 0.2245 with a P value of 0.089 which was statistically insignificant. The mean difference in salivary flow rate between controls and type II diabetics was 0.0790 with a P value of 0.731 which was statistically insignificant.

The results of our study are in accordance with the results of the study done by **Arati S Panchbai(2010)[6] and Juaan Aiket Saveendra (2015)[11].**

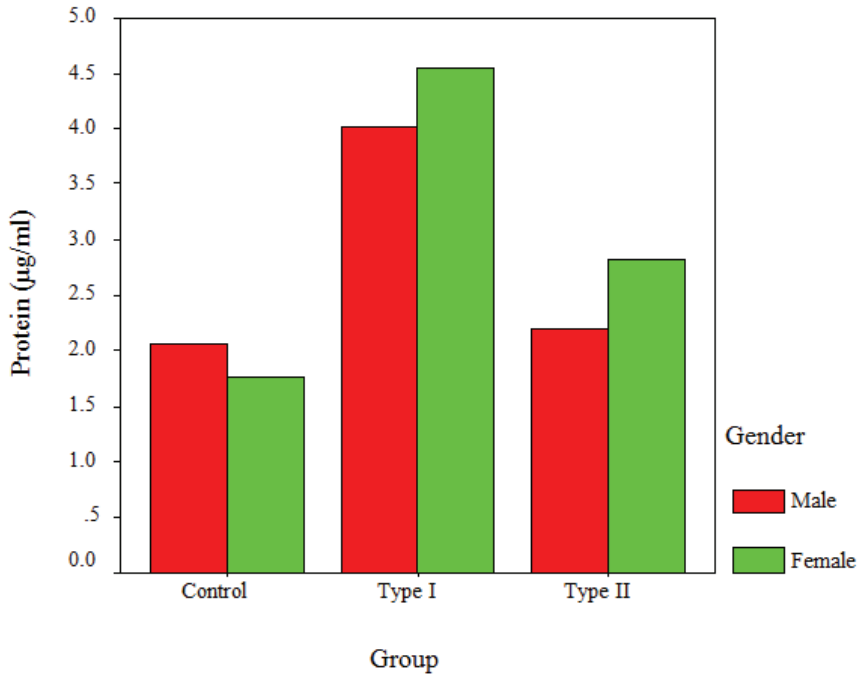
In the study done by **Arati S Panchbai (2010)[6],** in a total of 120 subjects, 40 in the uncontrolled diabetic, 40 in the controlled diabetic group and 40 in the healthy controls group, it was found that the P value was 0.56 which was not statistically significant. In a study done by **Juann Aiket Saveendra et al (2015)[11],** in a sample of 72 patients with type 2 diabetes, it was found that the P value was 0.518 which was not statistically significant.

The results of our study are in contradiction to the results of the study done by **Laisi T J et al (2012)[7]and Prathibha et al (2013)[9].**

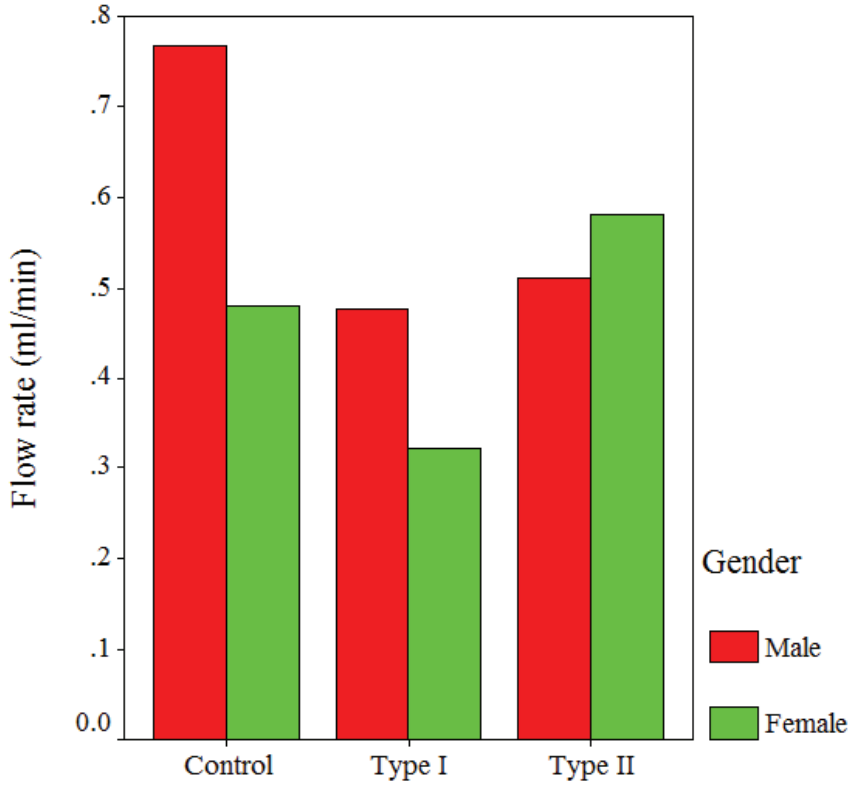
In the study performed by Laisi et al (2012) [7]to compare the salivary flow rate among diabetic and non diabetic subjects in a total of 40 subjects,the P value was 0.004 which was statistically significant. In a study performed by Prathibha et al (2013)[9] to compare the salivary flow rate among the diabetic and non diabetic subjects in a total of 60 subjects,the P value was 0.002 which was statistically significant.

The reason for such contradiction may be due to the fact that oral dryness occurring in diabetic patients could be multifactorial, either due to fatty infiltration of cells into the salivary glands, or physical alteration of mucosal cells subsequent to dehydration due to polyuria or microvascular disease, local inflammation and irritation in the oral cavity, infections, metabolic disturbances, and neuropathy affecting the salivary glands, and may be due to drug therapy for diabetes or concomitant drugs.

Graphs: Representation of the result in graphs:



Graph 1: COMPARISON OF TOTAL SALIVARY PROTEIN IN TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS IN MALE SUBJECTS AND FEMALE SUBJECTS:



Graph 2: COMPARISON OF TOTAL SALIVARY FLOW RATE IN TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS:

COMPARISON OF TOTAL SALIVARY PROTEIN AMONG TYPE I, TYPE II DIABETICS AND HEALTHY CONTROLS:

Multiple Comparison of total salivary proteins:

Dependent Variable: Protein (µg/ml)

Tukey HSD

Gender	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P value	95% Confidence Interval	
						Lower Bound	Upper Bound
Male	Control	Type I	-1.96046	.652936	0.015*	-3.57936	-.34156
	Control	Type II	-.13536	.652936	0.977	-1.75426	1.48354
	Type I	Type II	1.82510	.652936	0.025*	.20620	3.44400
Female	Control	Type I	-2.78661	.825046	0.006**	-4.83224	-.74098
	Control	Type II	-1.05711	.825046	0.418	-3.10275	.98852
	Type I	Type II	1.72950	.825046	0.110	-.31613	3.77513

* The mean difference is significant at the .05 level.

COMPARISON OF TOTAL SALIVARY FLOW RATE AMONG TYPE I, TYPE II DIABETICS AND HEALTHY CONROLS:

Multiple comparison of total salivary flow rate:

Dependent Variable: Flow rate (ml/min)

Tukey HSD

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control Type I	Type I	.2245	.10438	.089	-.0271	.4761
	Type II	.0790	.10438	.731	-.1726	.3306
	Control	-.2245	.10438	.089	-.4761	.0271
	Type II	-.1455	.10438	.351	-.3971	.1061
Type II	Control	-.0790	.10438	.731	-.3306	.1726
	Type I	.1455	.10438	.351	-.1061	.3971

Conclusion

A significant difference in total salivary protein level among the diabetic and non diabetics emphasized that protein utilization by other biochemical metabolic pathways has an overall systemic response to glucose intolerance. Insulin is known to have the potential to alter protein metabolism. Further many studies have confirmed that increase in whole salivary proteins was not associated with dental caries, except for the 17kDa protein which might be a risk marker for dental caries. Nevertheless, the overall results obtained from various studies regarding salivary proteins in diabetics remains controversial.

Various studies conducted to assess the salivary flow rate among diabetic patient have found to give inconsistent results, which may be attributed to the duration of diabetes, age range of patients and metabolic control of patients, class of drugs taken by the patient.

The present study has certain limitations like a small sample size, and hence the results are entirely conclusive, the study has provided a platform for further research.

Ethical Approval:

All authors at this moment declare that all the experiments have been approved by the appropriate ethics committee (Institutional Review Board, Ragas Dental College and Hospital, Chennai) and have therefore been performed by ethical standards.

Financial Support: Nil

Conflict of Interest: Nil

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