EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 5
587-592
Year: 2018

NON INVASIVE ESTIMATION OF SALIVARY GLUCOSE, SALIVARY AMYLASE, SALIVARY PROTEIN AND SALIVARY PH IN DIABETIC AND NON-DIABETIC PATIENTS - A CASE CONTROL STUDY

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Article Received on 27/02/2018 **Article Revised on 20/03/2018** **Article Accepted on 09/04/2018**

ABSTRACT

Introduction: Saliva deserves to be the best diagnostic fluid due to its simplified collection methods if proved successful in diagnostics at par with the serum. **Objective:** The objective of this study is to estimate and correlate the levels of salivary glucose, amylase, protein and ph in diabetic subjects and to assess if these salivary components can be potentially used as a non-invasive tool in diagnosing diabetes mellitus and monitoring glycemic control in diabetic patients. **Materials and Methods:** A total of 70 subjects aged between 35-65yrs participated in the study. Samples of unstimulated saliva was collected during the morning hours and their latest fasting plasma glucose levels were noted. **Results:** In this study there wasn't significant correlation between Salivary glucose and blood glucose. Except Salivary pH, all the other salivary parameters were found to be insignificant when compared between diabetic and non- diabetic groups. **Conclusion:** In our study we did not find a significant correlation between Salivary glucose and blood glucose. The salivary glucose should be compared with the fasting, post prandial and HbA1c to evaluate the standardised correlation.

KEYWORDS: Diabetes mellitus; Non-invasive; Salivary glucose; Salivary amylase.

INTRODUCTION

Diabetes mellitus is a common endocrine disorder characterised by relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues. Diabetes mellitus is a massive growing, silent epidemic that has the potential to cripple health services in all parts of the world. Asian Indians are at a greater risk of developing this disorder as per the literature. The prevalence rate of diabetes in urban areas is found to be about 9% and the prevalence has increased in rural areas to about 3% of the total population.[1]

Saliva is a biological fluid with multifactorial functional applications with regards to the maintenance of general health. The defence role of saliva is a proven function that is dealt by the imunoglobulins, lysozyme, peroxidase, cystatins, lactoferrin and hystatins inhibiting the growth of micro organisms.[2,3] Saliva has glandular (salivary glands) and non-glandular origin(fluids from oropharyngeal mucosae, crevicular fluid and also food debris and blood derived compounds).[4,5,6] Glandular salivary products vary in their concentration of salts/ions and total proteins based on their gland of origin. Proteins like Statherin, Proline rich proteins(PRP's) and mucins helps in maintaining the calcium content in the saliva.[7]

Saliva also plays a vital role in remineralisation of tooth enamel. Salivary proteins are expressed differently among individual glands. For example, Cystatin C is secreted by the submandibular gland and MUC 5B mucin and calgranulin are secreted by the sublingual gland.[8] The composition of the saliva also varies with rest and stimulation (stimulated/unstimulated saliva).

Plasma compounds can enter the saliva through various process. Albumin enters into the oral fluid by plasma leakage through transduction.[9] Amylase in the saliva is secreted from salivary gland. It helps in digestion of starch and has antimicrobial action. The concentration of alpha amylase in saliva is 3257 plus or minus 1632 U/ml and Albumin is 0.2 plus or minus 0.1 mg/ml. Saliva collection is an easy and non-invasive method and moreover more accepted by the patients when compared to serum collection, but the collection protocol such as the timing of collection, avoiding food and fluid (apart from water) for atleast 30 minutes before the collection should be adopted precisely to avoid errors in analysis results. There are different methods of saliva collection which includes passive drooling and spitting, of which spitting method is commonly used as it is comparatively easier than other methods but has a drawback of

contamination of bacteria while spitting directly into a collector vial.[10]

Saliva samples should be stored preferably on ice and frozen as soon as possible to maintain the sample integrity. Saliva naturally contains bacterial protease enzyme which can degrade several salivary proteins, therefore specific storage method influences the saliva compounds concentration which would in turn affect the analysis results.[10] Immediate storage of saliva at room temperature if the analysis is to be carried out immediately or within 30-90mins of collection does not cause degradation of salivary compounds.

A low pH in salivary analysis indicate that it is much more acidic than normal. Saliva collection has its own advantages like easy collection, non-invasive, can be done with limited training and do not require a special or specific tool. Salivary analysis for systemic disorders are usually done with whole saliva. Commonly the diagnostic procedures utilise the blood constituents for detecting diseases but saliva has more advantages than blood as a diagnostic tool as mentioned above. This is very applicable with children and elderly. The present study was arrived at Non-invasive estimation of Salivary Glucose, Salivary Amylase, Salivary protein and Salivary pH in Diabetic and Non-diabetic patients as Diabetes affects any age-group crippling the quality of life.

METHODOLODY

Ethical clearance: Ethical clearance was obtained by institutes ethical committee.

Collection of Sample: This is a case- control study with a study sample of 140 subjects including 70 known Diabetic as study group and 70 healthy individuals as control group. Both the groups were age and sex matched. Patients aged between 30-75 years those who have been already diagnosed with Diabetes Mellitus were included in the study. Exclusion criteria included those with decreased salivation, severe diabetic complications, with systemic diseases other than diabetes. Saliva sample collection was performed, after the patients gave a written consent [Annexure 1]. The samples were collected in the morning hours between 8:00-11:00 am with the patients sitting in a comfortable straight position in a composed manner. Samples were collected by the spitting method in a sterile container each for specific patients. Thus the unstimulated whole saliva was collected.

Methodology: Levels of Salivary Components were assessed at Chettinad Hospital Biochemistry Clinical Laboratory. Salivary glucose, proteins, amylase and pH were recorded for both the control and study groups. Comparison between the groups were done using independent samples ‘t’ test. SPSS version 20 was used for statistical analysis.

The collected salivary samples are pipetted out and centrifuged of about 2500rpm for five minutes. The samples were then taken in small PCR tubes and the required amount of reagent (1000 Ul for glucose, 500 Ul for amylase and protein) and salivary sample of about 10 Ul for glucose, 5 Ul for protein and 50 Ul for amylase was taken respectively. For glucose the mixed solution is incubated at 37 degree centigrade for 10 minutes and for amylase one minute and then processed. For protein estimation(Albumin) the mixed solution is not incubated. All the above parameters were processed one at a time in the analyzer and values are noted. For estimation of pH, pH strips are dipped into the salivary sample and measured with a scaling given by the manufacturer.

OBSERVATION AND RESULTS

The aim of the study was to evaluate saliva as a non-invasive tool for the estimation of Salivary Components. 140 patients were screened for this study, among that 70 patients were diagnosed diabetic and 70 patients served as control out of which both the groups where sex and age groups matched.

When Blood glucose and Salivary glucose was compared among the Diabetic and Non-diabetic group of 140 patients each [Table:1], and this data subjected to analysis was an insignificant value. (p- 0.636). Salivary glucose was then compared between the Diabetic and Non-diabetic group [Table 2;Fig 1], and this on statistical analysis was found to be non-significant. (p – 0.066).

Similarly, Salivary amylase was compared among the same 140 population of diabetic and non-diabetic [Table 2; Fig 2] and was found to be highly significant. (p-0.002).

Salivary protein and Salivary pH was compared [Table 2; Fig 3,4] which on data analysis was found to be Highly significant (p-0.000).

Correlation was made among grouping of age groups [Table 3] of the 140 patients into 30-45years; 46-60years; 61-75 years and was subjected to comparison on all the salivary parameters such as Salivary glucose (p value 0.349), Salivary amylase (p value 0.782), Salivary protein (p value 0.532) and Salivary pH (p value 0.940) which on analysis was all found insignificant.

The diabetic and non-diabetic group of patients were further separated based on their gender [Table 4] and was analyzed for the same salivary parameters of Glucose (p value 0.707), Amylase (p value 0.962), Protein (p value 0.874) and Ph (p value 0.052) were insignificant.

Table 1: This Figure Shows The Mean Values Of Salivary Glucose In Diabetic And Non-Diabetic Patients.

		Blood Glucose	Salivary Glucose
Blood Glucose	Pearson Correlation	1	-0.058
	Sig. (2-tailed)	.	0.636
	N	70	70
Salivary Glucose	Pearson Correlation	-0.058	1
	Sig. (2-tailed)	.636	.
	N	70	70

Table 2: Correlation Between Salivary Glucose, Amylase And Protein In Diabetic And Non-Diabetic Patients.

	Group	N	Mean	Std. Deviation	Std. Error Mean	p value
Salivary Glucose	Diabetic	70	3.621	8.7277	1.0432	0.066
	Non-Diabetic	70	1.632	2.1401	.2558	
Salivary Amylase	Diabetic	70	7.0070	10.06065	1.33257	0.002
	Non-Diabetic	70	1.4371	10.02470	1.19818	
Salivary Protein	Diabetic	70	.9806	1.15040	.13750	0.000
	Non-Diabetic	70	1.8117	.09253	.01106	
Salivary pH	Diabetic	70	6.60	.689	.082	0.000
	Non-Diabetic	70	7.01	.318	.038	

Table 3: Correlation Of All Parameters Between Three Age Groups.

	Age in years						
	30-45		46-60		61-75		p value
	Mean	SD	Mean	SD	Mean	SD	
Blood Glucose	163.38	47.56	156.43	48.95	153.00	23.92	0.833
Salivary Glucose	2.67	2.03	2.96	4.43	6.81	18.71	0.349
Salivary Amylase	8.19	11.48	7.19	10.33	5.23	8.19	0.782
Salivary Protein	1.31	1.12	.90	.75	.93	2.04	0.532
Salivary pH	6.62	.51	6.61	.72	6.54	.78	0.940

Table 4: Correlation Based on Gender of Diabetic Patients.

	Sex				P value
	Male		Female		
	Mean	SD	Mean	SD	
Blood Glucose	155.62	41.26	158.47	48.24	0.792
Salivary Glucose	3.21	3.76	4.01	11.69	0.707
Salivary Amylase	7.08	8.24	6.95	11.50	0.962
Salivary Protein	1.00	.90	.96	1.36	0.874
Salivary pH	6.76	.70	6.44	.65	0.052

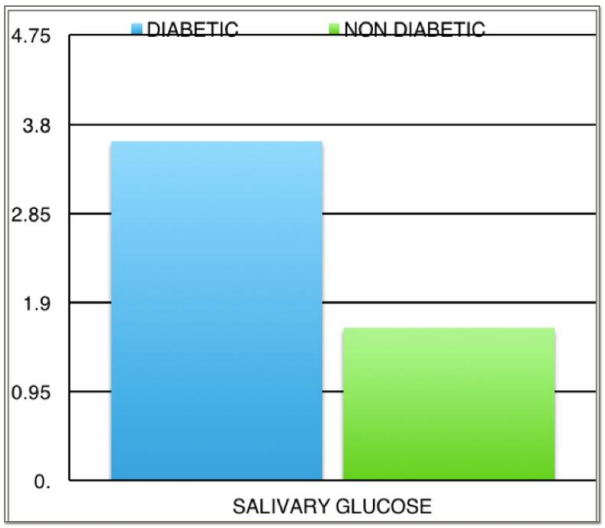

Fig 1: This figure shows the mean values of salivary glucose in diabetic and non-diabetic patients.

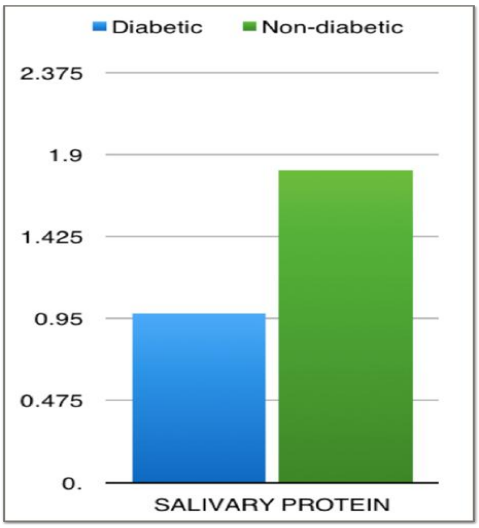

Fig 3: This figure shows the mean values of salivary albumin in diabetic and non-diabetic patients.

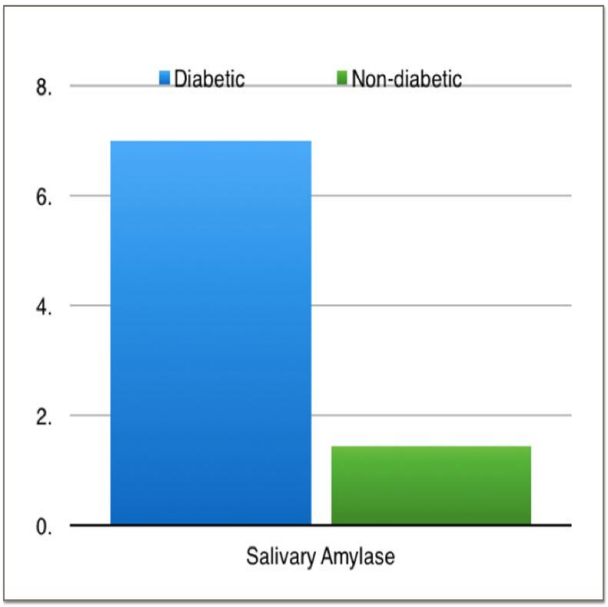

Fig 2: This figure shows the mean values of salivary amylase in diabetic and non-diabetic patients.

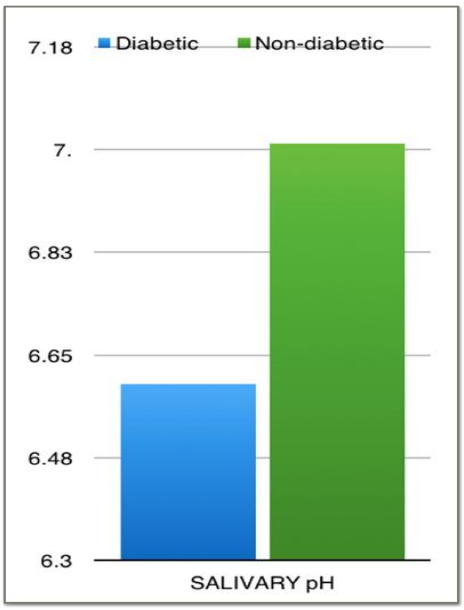

Fig 4: This figure shows the mean values of salivary pH in diabetic and non-diabetic patients.

DISCUSSION

In the present study, there was insignificance when salivary glucose and serum glucose were compared which is in accordance with Forbat et al, Carda et al, Jurysta et al.[11,12,13] The results of our study was in contradiction with the study conducted by Arathy S. Panchbai[14] wherein they had a significant positive correlation between salivary glucose and fasting blood glucose level. This contradiction might be because of the longer duration of disease in our study population which is in accordance to the report given by Arati S. Panchbai that, shorter disease duration tends to be associated with higher salivary glucose when compared with longer disease duration.[14]

The results were also in contradiction to the study conducted by Satish BN et al and Amer S et al and Mascarenhas P et al wherein they had compared the salivary glucose correlation with HbA1c values which is unlike our study where we have compared salivary glucose with fasting blood glucose.[1,15,16]

When salivary glucose concentration was compared between patients with diabetes mellitus and healthy controls there was a insignificant difference which is in accordance to the study conducted by Marchetti et al[17] and is in contradiction to the study conducted by Carda et al[12], Aydin et al[18], Shashikumar and Kannan[19], Vasconcelas et al[20], Panda abikshyeetetal et al[21], Panchbai et al[14], which could be attributed to the study sample where we had not sub-grouped them as controlled and uncontrolled diabetic patients and moreover this variation between the studies regarding salivary glucose level could be due to difference in the concentration of the compounds entering from the plasma into saliva and this could have compromised the quantitative estimation of salivary compounds.

When salivary amylase was compared with diabetic and non- diabetic groups there was a significant increase(p=.002) which is similar to the results obtained by the study done by Paul et al and Meurman et al[22], Dodds MW et al[23], Aydin et al.[12] The increase in salivary amylase in diabetic patients could be because of greater expression of amylase and cyclic amp receptor in parotid gland of diabetic patients than that of non - diabetic which was demonstrated by Piras et al.[24]

With regard to salivary protein, the present study results does not provide significant differences between study and control group and is similar to the study conducted by Arati S Panchbai.[14] in 2010. However, recent studies conducted by Pal P and Desai NT[25]; Lopez ME and colloca ME[26] and Yavuzyilmaz E, Streckfus et al[27] have reported higher salivary total protein levels in diabetics and this could be due to basement membranopathy is higher in diabetic patients making it pre locatable of the increase of protein content in saliva and is in contradiction to our study and the reason could be possibly due to our study pertaining only to Albumin protein and not total protein. In agreement to our study result, Arun A. Sre Kennath J et al also reported significantly lower salivary pH (acidic) in type 2 diabetic patients when compared to normal subjects. He also stated that salivary pH value become acidic as diseases state progressed from Non-diabetic to diabetic. In our study we also found that salivary pH is significantly low in men when compared between male and female in diabetic groups which could be due to effect of smoking in men which might alter the salivary pH.

When salivary Amylase and Salivary glucose were compared between male and females among diabetic group, there was no significant difference. In our study there was no significant difference in values compared between the age groups in the salivary samples.

CONCLUSION

In our study we did not find a significant correlation between Salivary glucose and blood glucose whereas multiple studies reported a significant correlation. This might be because we did not subgroup diabetic study population into controlled and uncontrolled further, the duration of the disease also plays a major role in support with the correlation. The transfer of serum constituents into saliva is related to the physiochemical characteristics of these molecules. Further more different substances reach saliva by different mechanism. Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary pH. In addition, many serum markers can reach saliva in a unpredictable way (GCF flow and through oral wounds), these parameters will affect the diagnostic usefulness of many salivary constituents [FDI working group 10,1992]. The intake of medications may affect the salivary gland function which in turn might affect the composition of saliva. So, these diabetic groups should also be sub grouped based on the duration of the disease and moreover, the salivary glucose should be compared with the fasting, post prandial and HbA1c to evaluate the standardized correlation.

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