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Quantitative Estimation of Salivary Biomarkers in Diabetic and Non-Diabetic Individuals

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Abstract
Background: *Saliva as a diagnostic tool, is gaining attention world-wide. The value of saliva as an indicator of systemic disease has also been explored. Oral physicians hold the responsibility of recognizing significant associations between certain oral anomalies and diabetes mellitus. The aim of the present study is to assessed the salivary levels of amylase, albumin, glucose, total protein and c-reactive protein in individuals having chronic periodontitis with and without diabetes.*
Material & Methods: *Patients visiting the department of Periodontology on an out-patient basis at Darshan Dental College and Hospital, Udaipur, Rajasthan were included in the study. Total 26 patients, 13 patients with chronic periodontitis with type II diabetes (group A), 13 patients with chronic periodontitis without diabetes (group B) were included in the study after obtaining written consent. After periodontal recordings and Hba1c reports, Saliva sample was collected from patients at baseline for evaluation of salivary amylase, albumin, total protein, c-reactive protein and glucose levels.*
Results: *Our study showed that the mean value of glucose, total protein and albumin in saliva was higher in group A as compared to group B, which was statistically significant (P<0.05. P<0.01 & P<0.05 respectively). The mean value of C-reactive protein and amylase was slightly higher in group A as compared to group B, which was statistically non- significant (P=1.26 & P=0.34 respectively.*
Conclusion: *Diabetes mellitus has been consistently documented to be associated with altered salivary composition and function. Although many studies have been carried out on salivary parameters in diabetes, our study will be one of the very few that will assess all the salient parameters and variables affecting them in a single cohort.*
Keywords: *Diabetes mellitus, Salivary biomarkers, HbA1c, Periodontitis.*

Introduction

Diabetes mellitus is a syndrome or set of syndromes resulting from an absolute or relative insulin deficit. It is estimated that around the turn of the century, there were 40 million diabetics in India, and worldwide, the prevalence of diabetes is thought to have doubled between 1994 and 2010, with about 240 million people now suffering from the disease. 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.[1]

The value of saliva as an indicator of systemic disease has also been explored. Mandel and his co-workers have collected an enormous amount of data on salivary changes in disease over many years. Saliva biomarkers display unique patterns in Diabetics.[2] Oral fluid or whole saliva is a complex chemical milieu of teeth and oral soft tissues, consisting mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and numerous other important constituents like glucose and amylase.

Evidence has consistently indicated that diabetes is a risk factor for increased severity of gingivitis and periodontitis. Conversely, periodontitis may be a risk factor for worsening glycemic control among patients with diabetes and may increase the risk of diabetic complications. Periodontitis may initiate or propagate insulin resistance in a manner similar to that of obesity, by enhancing activation of the overall systemic immune response initiated by cytokines. Given these mechanisms promoting insulin resistance, it seems that in individuals with type 2 diabetes and periodontitis, an elevated chronic systemic inflammatory state induced by periodontal disease may contribute to insulin resistance through a feed-forward mechanism, worsening glycemic control. This might explain why periodontitis increases the risk of poor glycemic control among patients with type 2 diabetes.[3]

Very few studies have been performed on salivary composition and function in diabetes, particularly in India; thus, the data to date are limited. Furthermore, the study results that have been reported are often contradictory in several aspects, and this suggests the need for further investigative studies. The relative inconsistency in the outcomes of various studies may be attributed to variations in the duration of diabetes, the age range of patients, and the metabolic control of diabetes. The potential of saliva to aid in the monitoring of diabetes mellitus was therefore examined in the present study. Of all salivary parameters, glucose, C-reactive protein, albumin and salivary flow rate appear to be the most closely related to the oral environment in diabetic patients.[4]

Saliva as a diagnostic tool, is gaining attention world-wide. Due to non-invasiveness of its collection and non-necessity of skilled technician for collection, and presence of sensitive biomarkers in saliva, it is considered as a worthwhile diagnostic tool. The aim of the present study is to assessed the salivary levels of amylase, albumin, glucose, total protein and c-reactive protein in individuals having chronic periodontitis with and without diabetes.

Material & Methods

Patients visiting the department of Periodontology on an out-patient basis at Darshan Dental College and Hospital, Udaipur, Rajasthan were included in the study. Total 26 patients, 13 patients with chronic periodontitis with type II diabetes (group A), 13 patients with chronic periodontitis without diabetes (group B) were included in the study after obtaining written consent.

Inclusion criteria for Group A

1. Patients with chronic periodontitis with age between 35-70 years of age.
2. Presence of type 2 diabetes, criteria for diagnosis of Diabetes mellitus-symptoms of diabetes plus HbA1c > 6.0%.

3. Presence of >10 teeth per dental arch, excluding third molars.
4. No modification in diabetic medication in two months before or during the study.
5. Clinical attachment level≥2mm
6. Pocket depth≥5mm

Inclusion criteria for Group B
7. Patients with chronic periodontitis with range 35-70 years of age.
8. Probing depth more than or equal to 5mm.
9. Attachment loss more than or equal to 2mm.
10. Radiographic evidence of bone loss.

Exclusion Criteria
1. Patients with any systemic diseases which are known to affect any of the considered parameters in any way.
2. Smokers.
3. Pregnant and lactating females.
4. Any periodontal therapy in the previous 6 months.
5. Use of any antimicrobials in the previous 3 months.
6. Any other infections (eg. Common cold, influenza, any other ENT infections etc.) which may affect any of the considered study parameters.
7. Radiation therapy.
8. Long standing infections.

Clinical parameters
1. Pocket depth measured at 6 sites- 3 buccal (mesiobuccal, midbuccal and distobuccal) and 3 lingual (mesiolingual, midlingual and distolingual) sites with a UNC- 15 periodontal probe.
2. Clinical attachment level (CAL).
3. Gingival Bleeding Index (GBI) (Ainamo and Bay).
4. Panoramic radiograph and Intra Oral Periapical radiographs– for radiographic alveolar bone amount.
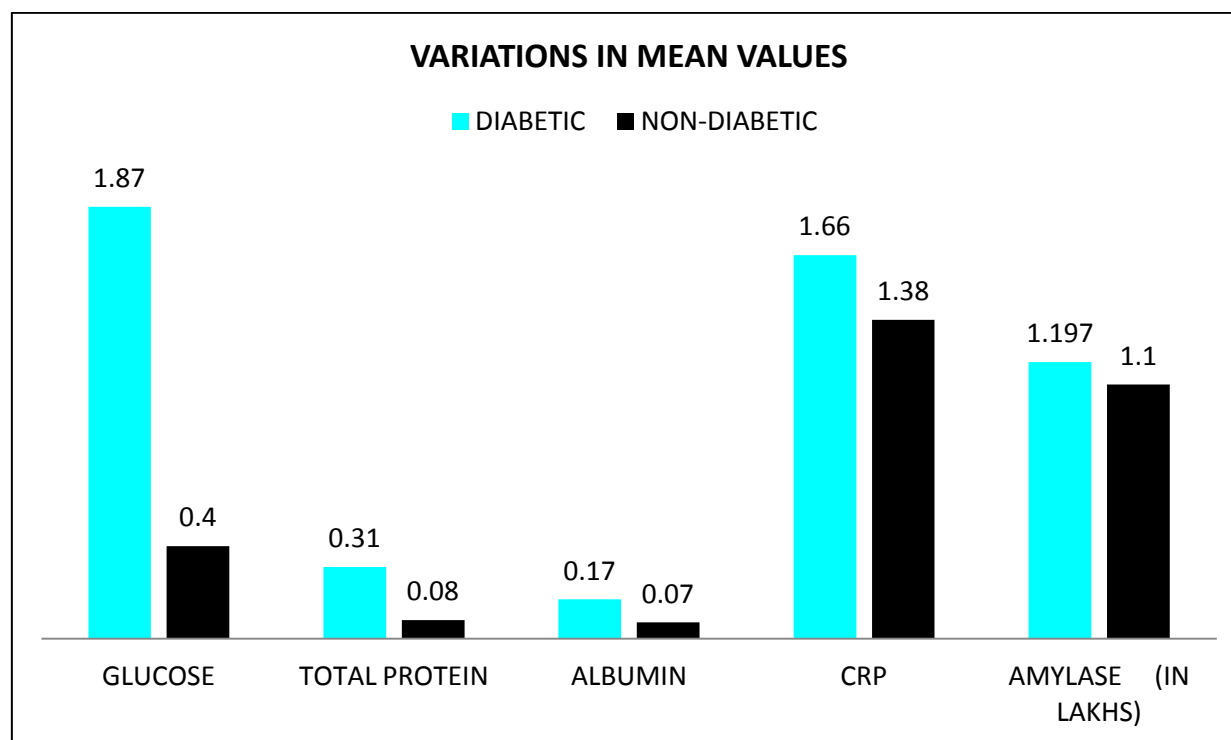
Collection of saliva
After periodontal recordings and Hba1c reports, saliva samples were collected from all patients at baseline from (8:30 to 10:30) following a brief rinse with water 10 minutes prior to sample collection. 30 milliliters of unstimulated whole saliva was collected by spitting method from subjects by instructing them to expectorate into a sterile container and was then stored at 4° C temperature. The containers with the samples were placed in a Styrofoam box with the ice and sent to the biochemistry laboratory of Darshan Dental College and Hospital for the salivary test.
Freezing saliva samples will precipitate mucins. On day of assay, thaw completely, vortex, and centrifuge at 1500 x g (@3000 rpm) for 15 minutes. Centrifuging removes mucins and other particulate matter which may interfere with the assay. Saliva sample was collected from patients at baseline for evaluation of salivary amylase, albumin, total protein, c-reactive protein, and glucose levels.

Results
Our study showed that the mean value of glucose, total protein and albumin in saliva was higher in group A as compared to group B, which was statistically significant (P<0.05. P<0.01 & P<0.05 respectively). The mean value of C-reactive protein and amylase was slightly higher in group A as compared to group B, which was statistically non- significant (P=1.26 & P=0.34 respectively.

Table 1: Comparison of Different salivary biomarkers in between groups

		Glucose (mg /dl)	Total protein (gm/dl)	Albumin (gm/dl)	C- RP (mg/L)	Amylase (U/L)
DIABETIC	Mean	1.87	0.31	0.17	1.66	119783.33
	SD	3.02	0.26	0.14	0.78	93348.83
NON DIABETIC	Mean	0.40	0.08	0.07	1.38	110057.92
	SD	0.37	0.05	0.04	0.22	49032.78
	t value	1.80	3.26	2.55	1.26	0.34
	P Value	<0.05	<0.01	<0.05	NS	NS

Graph 1: Variations of mean value in both groups

Discussion

The diagnosis of DM is established by determining the concentration of glucose in blood serum using standardized methods. However, monitoring blood glucose levels at frequent intervals causes discomfort for patients during the procedure of venipuncture, why many patients do not regularly monitor blood glucose concentration. In the recent years, efforts have been made to replace blood test with other biological material samples that could be collected by noninvasive procedure. One of these samples can certainly be saliva. It has many advantages over serum, such as inexpensive and non-invasive collection procedure, including ease of storage and delivery. Saliva is body fluid with complex composition and specific roles. The analysis of biochemical constituents in saliva is of great help in diagnostics of diseases in oral cavity as well as monitoring general health of organism.

Whole saliva contains locally produced substances as well as serum components that can be used for diagnosis of a variety of systemic diseases and understanding of their oral manifestations.[5] Mata et al reported alterations of salivary composition in diabetic patients. These biologic changes in diabetic whole saliva were different from one study to another that may be due to the diversity in sample selection criteria and study design. Based on numerous studies, it has been proved that there is a modification of organic and inorganic constituents of saliva in diabetic patients [6].

The present study showed that the mean *salivary glucose* levels were found to be significantly elevated in both uncontrolled and controlled diabetics, as compared to healthy non-diabetics which was similar to a study by Arathi s et al.[3] and other studies also have similar results. The salivary albumin of type 2 diabetic patients was significantly higher in comparison with control group. These results are in agreement with reports of Ben-Arch[7] and Dodd's et al[8]. This suggests that low grade infection of the salivary glands causing increased leakage of serum proteins into the saliva that is common finding in diabetic patients.

Although, Meurman et al.[9], Carda et al.[10] and Collin et al.[11] reported that no difference in salivary albumin was found between type 2 diabetic patients and control group. This conflict could be explained by the different type of saliva, stages of the disease and metabolic control status of the patients.

Our study showed that statistically significant difference in salivary total protein was found among the groups examined. Some studies have reported similar total salivary protein concentrations in diabetic and control groups (Harrison et al., 1987[12]; Belazi et al., 1998[13]) although others have found salivary protein levels from diabetics to be either higher (Lopez et al., 2003)[14] or lower (Streckfus et al., 1994[15]; Yavuzyilmaz et al., 1996[16]). These conflicts could be explained by the fact that the different studies examined type II DIABETIC patients in different disease stages.

The other parameters such as, C-RP, and amylase were not statistically significant but as our study is still an ongoing study we our still expecting further change in the final data and cannot draw any conclusive result. The relatively small sample size (13 type 2 diabetic patients and 13 non-diabetic patients) adds value to the statistically significant result observed.

Conclusion

Diabetes mellitus has been consistently documented to be associated with altered salivary composition and function. Oral physicians hold the responsibility of recognizing significant associations between certain oral anomalies and diabetes mellitus. Although many studies have been carried out on salivary parameters in diabetes, our study will be one of the very few that will assess all the salient parameters and variables affecting them in a single cohort.

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