Original Article

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Evaluation of Salivary Profile among Adult Type 2 Diabetes Mellitus Patients in South India

Physiology Section

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ABSTRACT

Background: A lack of consensus on the possible association between diabetes and salivary dysfunction motivated us to conduct this investigation on the salivary parameters in diabetic and non diabetic subjects. This could also make the use of saliva as an alternative to that of blood in the diagnosis/monitoring of diabetes mellitus.

Objectives: To compare the salivary flow rates and the salivary physical and biochemical parameters of diabetic (D) and non diabetic (ND) subjects.

Material and Methods: The participants in this study included 30 non diabetic subjects and 30 diabetic volunteers who had Type 2 Diabetes mellitus for a minimum of 2 years. Unstimulated whole saliva was collected in the fasting state. Salivary pH, flow rate and organic and inorganic constituents were evaluated. Data which was collected was statistically analysed and interpreted.

Results: Salivary pH (ND=7.09±0.29, D=6.69±0.35), flow rate (ND=0.67±0.07, D=0.46±0.02) and salivary amylase (ND=92.51±13.74, D=19.20±1.8) were significantly lower in diabetics. They had significantly higher levels of salivary glucose (ND=4.33 ± 0.29, D=17.31±2.05), total proteins (ND=424.46±237.34, D=877.29±603.84), sodium (ND=4.31±0.65, D=14.42±1.83) and potassium (ND=20.84±0.71, D=25.95±1.56) and lower levels of calcium (ND=6.39±0.5, D=4.22±0.12) in comparison to those in the non-diabetic group.

Conclusion: Significant variations were observed in salivary physical and biochemical parameters between diabetics and non diabetics. Evaluation of salivary parameters can be a cost effective and a non invasive alternative for screening, diagnosis and monitoring of diabetes, to blood.

Key words: Saliva, Type 2 Diabetes mellitus (T2DM), Salivary flow rate, Salivary glucose

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a treatable but a chronic condition and the main risks to health are its characteristic long-term complications. Epidemiological studies, worldwide and in India, show a significant increase in the burden of T2DM. Global estimates for the prevalence of diabetes as have been put forth by J E Shaw et al., predict the prevalence of diabetes among adults (aged 20–79 years) to reach 7.7% (439 million adults) by 2030 [1].India and other south Asian countries are also not exceptions to this trend. The prevalence of obesity and the metabolic syndrome is rapidly increasing in turn, leading to increased morbidity and mortality which are caused by T2DM and cardiovascular disease (CVD) [2]. DM frequently leads to hormonal, micro–vascular and neuronal changes as a result of metabolic dysregulation, which often compromises the ability of the multiple organ systems in functioning [3]. Prolonged hyperglycaemia in T2DM can compromise the immune, cardiovascular, renal and ophthalmic systems, thus producing an array of complications like neuropathy, peripheral vascular disease, renal disease, retinopathy and coronary heart disease.

In addition, it has been noted that the salivary glands are also affected directly or indirectly. Reported oral health complications which are associated with T2DM, which are usually encountered by practitioners include xerostomia, tooth loss, gingivitis, periodontitis, odontogenic abscesses and soft tissue lesions of the tongue and the oral mucosa [4]. Multiple physiologic factors contribute to compromised salivary function in poorly controlled T2DM. Diabetes-associated autonomic neuropathies, microvascular changes, hormonal imbalances or a combination of these are responsible for salivary hypo function and dehydration in diabetics [5].

However, there is no consensus on the possible association between T2DM and salivary dysfunction in diabetes. This study was planned to evaluate the hypothesis that salivary parameters, both physical and biochemical, varied between the diabetic and non diabetic subjects. The objectives of this study were, to compare the salivary flow rates and physical and biochemical parameters in diabetic and non diabetic subjects and to evaluate the correlation between fasting plasma glucose levels and salivary parameters.

MATERIAL AND METHODS

The participants in this study were 30 diabetic and 30 non diabetic subjects. The subjects were enrolled on a convenient sampling basis (30 subjects in each group, as it was a small scale pilot study). Cases were subjects of both sexes, who were 40 –55 years of age, who were suffering from diabetes for a minimum two years and were attending the laboratory of a private hospital in Chennai, Tamil Nadu, India. Available evidence suggests that the complications which are related to DM develop from the first year after the development of the disease [6]. Hence, the enrolled cases had a history of T2DM which had a minimum duration of 2 years. People who were on treatment with antidepressants, antihistaminics and anti hypertensives, those who were edentulous or had any systemic illnesses and those who were undergoing radiotherapy to head and neck region were excluded from the study [7–9]. The controls included non diabetic patients who attended the OPD and laboratory for other investigations. The study was approved by the Institutional Ethics Committee of our University. Written informed consents were obtained from all the participants before they were enrolled into the study.

Questionnaires which requested details of demographic data and medical history were administered to the subjects, following which saliva was collected from them in the morning between 7 am–8.30 am in the fasting state. Unstimulated whole saliva was collected by means of the standardised spitting technique, for 5 minutes. A single observer collected all the saliva samples from the subjects, to eliminate any chance of a bias. Saliva samples were transported within an hour and the salivary pH was immediately measured by using glass electrodes of Systronics μ pH system 361.

Salivary flow rate was calculated and it was expressed as milliliters per minute.

Samples were centrifuged at 5000 rpm, the supernatants were collected and they were stored at – 800°C until further analysis. Biochemical parameters in saliva were also analysed, which included organic constituents like salivary glucose (analysed by glucose kit method), salivary alpha amylase, salivary total proteins (analysed by Lowry's method) and inorganic constituents like sodium, calcium and potassium (analysed by using a semi auto analyzer). Statistical analysis was performed by using SPSS, version 15. Salivary parameters were compared between diabetics and non diabetics by using Student's t test.

RESULTS

In the present study, 60 subjects (30 diabetic cases and 30 non diabetic controls) who were in the age range of 40 – 55 years were enrolled in the study. The mean age of the participants in the study was 46.5 years. Among the diabetics, there were 16 males and 14 females with a mean age of 48.14 years. Among the non diabetics, there were 14 males and 16 females with a mean age of 44.44 years. No significant differences were observed between the sexes in both groups in pH, salivary flow rates and biochemical parameters.

[Table/Fig-1] shows the mean ± SD of salivary pH and resting unstimulated whole salivary flow rates for non-diabetics and diabetics. Salivary pH was observed to be significantly lower in diabetics as compared to that to non diabetics. (ND=7.09±0.29, D=6.69±0.35,p<0.0001). Flow rate was significantly diminished in diabetics (ND=0.67±0.07, D=0.46±0.02, p<0.01). Biochemical determinations showed significant differences between non–diabetics and diabetics [Table/Fig-2]. The study results showed significantly increased levels of salivary glucose, total proteins, sodium and potassium and decreased levels of calcium in diabetics (p<0.0001). A statistically significant decrease was noticed in the salivary amylase concentrations in diabetics (p<0.0001).

Parameter Studied	Non Diabetic Subjects (MEAN ± SD)	Diabetic Subjects (MEAN± SD)	p value
Salivary p H	7.09 ± 0.29	6.69 ± 0.35*	p=0.000
Salivary flow rate(ml/min)	0.67 ± 0.07	0.46 ± 0.02*	p=0.002

[Table/Fig-1]: Salivary Physical Parameters In Non-Diabetic & Diabetic Subjects
(* - p < 0.05 was considered to be statistically significant)

Parameter Studied	Non-Diabetic Subjects (Mean ± Sd)	Diabetic Subjects (Mean ± SD)	p Value
Glucose (mg/dl)	4.33 ± 0.29	17.31 ± 2.05*	p=0.000
Salivary α amylase (nm maltose liberated /mt/mg protein)	92.51 ± 13.74	19.20 ± 1.8*	p=0.005
Total proteins(g/l)	424.46 ± 237.34	877.29 ± 603.84*	p=0.000
Sodium(mEq/l)	4.31 ± 0.65	14.42 ± 1.83*	p=0.000
Potassium(mEq/l)	20.84 ± 0.71	25.95 ± 1.56*	p=0.000
Calcium(mEq/l)	6.39 ± 0.5	4.22 ± 0.12*	p=0.000

[Table/Fig-2]: Salivary Biochemical Alterations In Non-Diabetic & Diabetic Subjects
(* - p < 0.05 was considered to be statistically significant)

DISCUSSION

Salivary parameters are altered by metabolic, nutritional and neurological abnormalities, the hydration status of a person and by drugs like anticholinergics, diuretics, antihistaminics, anti hypertensives, etc. [10] Diabetes is associated with microvascular complications and hence, autonomic neuropathy, both of which may affect the salivary secretions [11]. But the knowledge on the effect of T2DM on salivary functions remains equivocal, in spite of several studies which have been conducted in this regards. Hence, we planned to do this study on a south Indian diabetic population, to investigate whether the salivary physical and biochemical characteristics would be altered in diabetics and to compare them with those of non diabetic controls. Further, it was intended to propose the possibility of using saliva as an alternative to blood in the diagnosis/monitoring of diabetes mellitus.

Several studies which were done on resting salivary pH estimated a range of 5.5 – 7.9 in normal individuals [12]. The pH of saliva is maintained by carbonic acid and bicarbonate system, phosphate system and protein system of buffers [13]. This study showed a significant decrease in pH in diabetics in comparison with that in non diabetic subjects. Acidic pH was also observed in diabetic subjects by M E Lopez et al., in their study and this was attributed to either the microbial activity or a decrease in bicarbonate, which had occurred along with the flow rate [14]. Nevertheless, not much literature which pertains to salivary pH changes in T2DM is available.

Resting saliva is the mixture of secretions which enter the mouth in the absence of exogenous stimuli. Normal resting whole saliva flow rates range from 0.3 to 0.5 ml/min, whereas hyposalivation with symptoms of dry mouth appear in the range of 0.10 to 0.01 ml/min. Citric acid stimulated whole saliva flow rates are normally measured at 1.0 to 3.0 ml/min [15]. Salivary flow rate was significantly diminished in diabetics as compared to that in non-diabetics. The fact that the salivary flow rate was decreased in diabetics was in concurrence with the results of many other studies too [14,16].It can be explained that the thirst and dry mouth characteristic of diabetics was related to the poor glycaemic control in diabetics, which in turn, was associated with increased diuresis and fluid loss. This finding was also observed by Cherry–Peppers et al., in his study on salivary flow rates, in subjects without diabetes and in subjects with well-controlled type II diabetes [17].

Elisa M. Chavez et al., in their study, noticed that children with poorly controlled type 1 diabetes mellitus had decreased salivary flow rates in comparison to children with good glycaemic control and nondiabetic subjects [5]. Further, studies have shown restoration of normal salivary flow rates, once the glucose levels are well-controlled [10,18]. As the salivary secretion is regulated by the autonomic nervous system, the altered salivary secretion which is observed in the diabetic patients might also be related to diabetic autonomic neuropathy. However, in the studies of Meurman et al., and Tenovuo et al., the resting and stimulated, rate of salivary secretions did not differ between the T2DM patients and the controls [19, 20].

In this study, the salivary glucose level was significantly elevated in diabetics. This could be attributed to the altered glucose homeo-stasis. It was stated by Chatterton RT et al., that salivary glands act as filters of blood glucose, that would be altered by hormonal or neural regulations [21]. Many other authors have also found higher glucose salivary levels in diabetic patients than in non-diabetics [22]. Lopez et al., in their study, also observed that diabetic saliva glucose values were higher than those of the controls and also a negative correlation was found between salivary glucose levels and the glycaemic status and Hb A1 c levels of the subject [14].

The study results were also in agreement with those of Karjalainen et al., who also reported that the elevated salivary glucose levels in diabetics decreased after starting with insulin treatment [16]. On the contrary, Sharon et al., did not report any difference in salivary glucose levels [23].

Salivary α amylase levels were significantly lowered in diabetics than in non–diabetics. Our study results were concurrent with those of Kim et al., who found that both parotid gland amylase and its mRNA were reduced in streptozotocin–treated rats and that insulin treatment increased the amylase content before any effect on its mRNA occurred [24]. This result suggested an early effect

on protein translation and possibly, a long-term transcriptional effect. Dodds and Dodds associated the elevated amylase activity in his study with taste alterations in poorly controlled non-insulin-dependent diabetes mellitus adults [25]. No consensus is observed regarding the salivary amylase levels in diabetics. While Yavuzyilmaz et al., and Chatterton RT et al., reported that salivary α amylase values were higher in the diabetic group than in controls. They attributed this difference to stress and no differences were observed between the study groups with regards to the amylase activity by J. Tenouvo et al., [20,21,26].

The present study's results showed significantly increased levels of salivary total proteins in diabetics. The increase in salivary protein values can probably be attributed to greater microorganism activity or proteins of periodontal tissue origin. As proposed by Mandel, increased basement membrane permeability, which is often associated with diabetes, is one of the possibilities for the increased passage of proteins from the exocrine glands into their secretions in some patients [10]. Total salivary protein concentration, however, has been found to be similar in diabetic and control groups in some studies, although most of the studies have found salivary protein concentrations of diabetics to be higher [20]. Few studies have attributed the high levels of salivary proteins to the active periodontal disease which is commonly found in diabetics, wherein the proteins are derived from the gingival fluid and not the saliva [4].

Our study results showed that sodium and potassium were significantly elevated in diabetics but that calcium was significantly decreased. Significantly higher potassium concentrations were also observed by Ben Aryeh et al., in their studies on whole saliva of both T2DM and IDDM (Insulin Dependent Diabetes Mellitus) patients, in comparison to those in healthy controls [4]. The elevated potassium concentrations which were found in the diabetic patients may be due to either hyperaldosteronism or an impaired Na+-K+-ATPase activity, which lead to an altered transport of potassium in the salivary glands. Though a concurrence was observed with regards to the potassium levels, contradictory results were observed for the calcium levels by Harrison et al., [27].

Further, by assessing the magnitude of changes in the composition of saliva, an attempt to find out whether saliva could be used as an alternative to plasma in diagnosing or monitoring T2DM, was done. The most commonly used laboratory diagnostic procedures involve analyses of the cellular and chemical constituents of blood. Saliva offers some distinctive advantages over the other biological fluids. The collection and evaluation of the secretions from the individual salivary glands are primarily useful for the detection of the gland-specific pathology, i.e., infection and obstruction.

However, whole saliva is most frequently studied when a salivary analysis is used for the evaluation of systemic disorders. Some systemic diseases affect salivary glands directly or indirectly and they may influence the quantity as well as the composition of the saliva that is produced. These characteristic changes may contribute to the diagnosis and early detection of these diseases, as in the case of T2DM. Saliva is recently being used for the diagnosis of a wide range of diseases, as it has been proven to be an easily available, reliable and a non invasive diagnostic medium [10].

Whole saliva can be collected non-invasively and by individuals with limited training. No special equipment is needed for its collection . Diagnosis of a disease by doing the analysis of saliva is potentially valuable for children and older adults, since collection of the fluid is associated with fewer compliance problems in comparison to the collection of blood. Further, the analysis of saliva seems to be a cost-effective approach in the screening of large populations.

The present study has a few limitations, like a small sample size which prevented us from arriving at conclusions on the alteration in salivary parameters in diabetic subjects. A larger sample size would have helped us in establishing the relationship between fasting plasma glucose levels and various salivary parameters, particularly glucose. Also, it would have been a value addition if the status of the glycaemic controls of the subjects had been analysed by using HbA1c values.

CONCLUSION

This study showed significant variations in both physical and biochemical parameters of saliva in T2DM, thereby emphasizing the fact that the salivary composition was not just a reflection of the oral health state of a subject, but also of one's systemic state. Future studies can be conducted on a larger scale, taking into account the various limitations. This would help us in conclusively establishing the role of saliva in the screening and diagnosis of diabetes, as an alternative to blood.

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