Original Article

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Saliva as a Non-invasive Tool in Evaluation of Type 2 Diabetes Mellitus

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Abstract

Introduction: Diabetes mellitus is a chronic metabolic disorder and a leading cause of mortality and morbidity globally with an estimated prevalence of 70 million by 2025.

Materials and Methods: In total, 75 persons were included in this study. They were divided into 3 groups, each group consisting of 25 persons. Cases were selected from both the sexes and of age 40-70 years. Group I: Non-diabetes persons (controls), Group II: Controlled diabetes, Group III: Uncontrolled diabetes. All individuals were subjected to collection of blood and saliva for estimation of Glycosylated hemoglobin, fasting glucose, electrolytes, salivary pH and flow rate.

Results: In this study, fasting serum glucose and salivary glucose levels are comparable to other studies and shows statistical significance (P = 0.005), and hence, it can be utilized to evaluate glucose levels in diabetics. Regarding electrolytes, salivary calcium was decreased in uncontrolled diabetes (Group III) when compared to non-diabetics and controlled diabetics, whereas sodium and potassium showed no significant difference. Salivary pH and flow rate did not show any significant difference between the groups, whereas body mass index, waist circumference shows a statistically significant increase in uncontrolled diabetes.

Conclusion: This study gives evidence that saliva can be utilized as a non-invasive tool for evaluation of glucose in type 2 diabetes mellitus patients. In this study even though salivary calcium is significantly lower in uncontrolled diabetes, this cannot be taken as a marker for uncontrolled diabetes since this could be due to other confounding factors.

Key words: Body mass index, Diabetes mellitus, Fasting glucose, Waist circumference

INTRODUCTION

Diabetes mellitus is a clinically and genetically heterogeneous group of disorders affecting the metabolism of carbohydrates, lipids, and proteins. It is a complex multisystem disorder characterized by a relative or absolute insufficiency of insulin secretion or concomitant resistance to metabolic action of insulin on target tissues. Currently, we have 40.9 million people suffering from diabetes and the predicted estimate by the year 2025 is around 70 million.

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The interest has been recently increasing in non-invasive diagnostic testing for glucose and other parameters. Tests based on saliva have made substantial roads into diagnosis. It has been noted from various studies that salivary glands are affected directly or indirectly in diabetic mellitus. The complications include xerostomia, tooth loss, gingivitis and periodontitis, odontogenic abscesses, soft tissue lesions of the tongue, and oral mucosa. Multiple physiological factors contribute to compromised salivary function. Autonomic neuropathies, microvascular changes, hormonal imbalances or a combination of these are responsible for salivary hypofunction and dehydration in diabetics. Evaluation of salivary parameters has been shown to be cost-effective and non-invasive for screening, diagnosis and monitoring of diabetes when compared to blood investigations which are painful and causes physical trauma and mental stress to patients, hence the need for this study.

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MATERIALS AND METHODS

This is a prospective, randomized, cross-sectional study. The study was conducted in Meenakshi Ammal Dental College and Hospital, Chennai and Ethical Clearance was obtained for the same. A total no of 75 patients were included in this study, in which 50 patients were suffering from type 2 diabetes mellitus (which included both controlled and uncontrolled diabetics) and 25 non-diabetes persons (controls). The study population included both the genders, with an age range of 40-70 years.

The study population will be divided into 3 groups:
Group 1: Non-diabetics (control)
Group II: Controlled diabetic patients
Group III: Uncontrolled diabetic patients.

Inclusion Criteria

- Voluntary participation,
- Age inclusion: 40-75 years, and
- Sex included both the genders.

Exclusion Criteria

- Patients suffering from type 1 diabetes mellitus, pregnancy, physically and mentally challenged patients, chronic renal failure, hyperthyroidism, pancreatitis and pancreatic cancer, hypercholesterolemia. Medications, such as steroids, tricyclic antidepressants, epinephrine, diuretics, estrogen, lithium, and salicylates, were also excluded.

All patients were explained in detail about this study, and informed consent was obtained in their native languages to prevent language bias and later was subjected to collection of saliva and blood examination.

- Unstimulated saliva of 2 ml for 5 min will be collected and salivary flow rate and pH, levels of glucose, sodium, potassium, and calcium levels will be evaluated.
- 8 ml of venous blood will be collected and subjected to fasting glucose estimation, glycosylated hemoglobin (HbA1C), sodium, potassium, and calcium.

The testing of both salivary and serum samples was done in aseptic conditions. After 8 ml of venous blood sample was obtained from each subject, blood was immediately transferred into vacutainers and processed. The unstimulated saliva of 2 ml was collected for about 5 min immediately after blood collection in pre-weighed containers and estimated for same parameters with salivary flow rate and pH in subjects from all the groups. pH was estimated through salivary digital pH meter model no 111, which gave accurate data. The specimens were analyzed in room temperature and were fed into automated analyzer for interpretation of the following parameters. Fasting blood glucose in serum and saliva, HbA1c was measured through the following methods: Electrochemical colorimeter using a glucosymeter (Freestyle Precision - Abbots Diabetes Care Inc.), yielding blood glucose in milligrams per deciliter (mg/dL), HbA1c: High-pressure liquid chromatography (D-10 hemoglobin testing system - Bio-Rad Inc.), which gives the % of HbA1c fraction. Electrolytes levels of sodium and potassium in both serum and saliva were measured by Roche 9180 electrolyte analyzer, whereas for salivary and serum calcium estimation Optima 1 from Labindia manufacturers was done and described in Meq/l.

The results thus got were tabulated, and statistical analysis was done with statistical methods such as scatter plot and ANOVA.

RESULTS

In this study, fasting serum glucose and salivary glucose levels are comparable to other studies and shows statistical significance ($P = 0.005$) and hence it can be utilized to evaluate glucose levels in diabetics (Figures 1 and 2). Regarding electrolytes, salivary calcium was decreased in uncontrolled diabetes (Group III) when compared

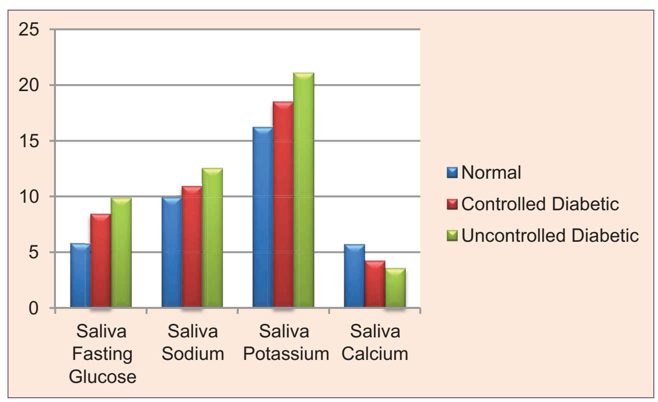

Figure 1: Salivary parameters in all groups

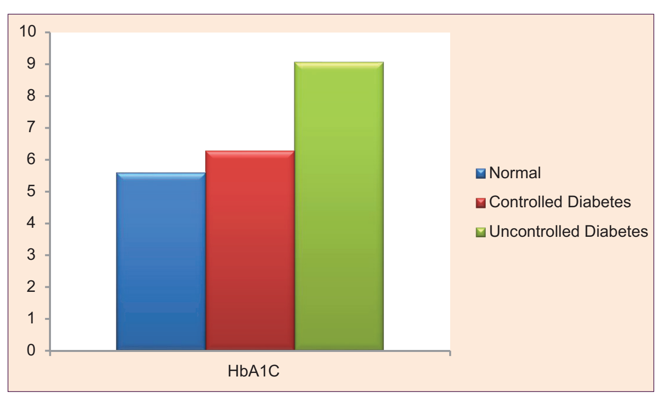

Figure 2: Glycosylated hemoglobin in serumin all groups

to non-diabetics and controlled diabetics, whereas sodium and potassium showed no significant difference (Figures 1, 3 and 4). Salivary pH and flow rate did not show any significant difference between the groups (Figures 5 and 6), whereas body mass index (BMI), waist circumference shows a statistically significant increase in uncontrolled diabetes (Figure 7).

DISCUSSION

Salivary parameters are altered by metabolic, nutritional, neurological abnormalities, the hydration status of a person and by drugs such as anticholinergics, diuretics, antihistamines, and antihypertensives.[1] Among the metabolic disorders, diabetes mellitus is the most significant disorder associated with varied oral manifestation ranging from xerostomia to serious bacterial and fungal infections leading to morbidity and mortality along with alterations in levels of glucose, electrolytes, pH and flow rates of saliva. This is due to salivary gland dysfunction because of microvascular complications and autonomic neuropathy, particlarly in uncontrolled diabetes. These parameters need to be assessed for the early detection and proper management of diabetic patients. Hence, we planned to do this study in a tertiary care hospital in South India to assess the possibility of utilizing saliva as a noninvasive alternative tool to blood in the monitoring of diabetes mellitus.

High salivary glucose level is a consequence of high plasma glucose level which diffuses in saliva, according to Lasisi *et al.*, 2002.[2] This high salivary glucose in conjunction with overall diminished flow of saliva has also been reported to be responsible for xerostomia in diabetic patients. This study showed a statistically significant correlation between saliva and serum glucose levels (fasting and HbA1c glucose levels) in uncontrolled diabetic patient group (P = 0.01 level) which was in concordance with studies conducted

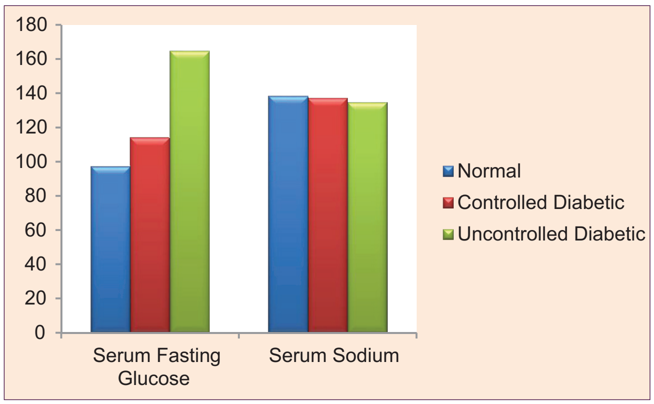

Figure 3: Serum parameters in all the 3 groups

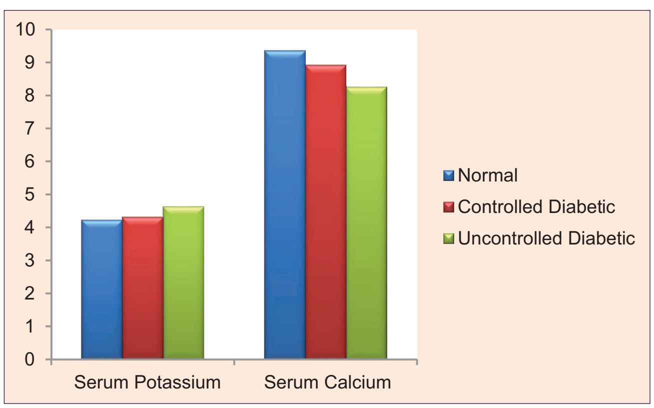

Figure 4: Salivary PH (Serum Potassium / Serum Calcium)

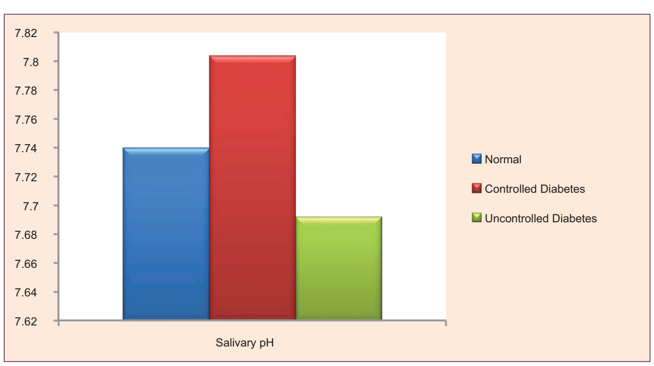

Figure 5: Salivary PH

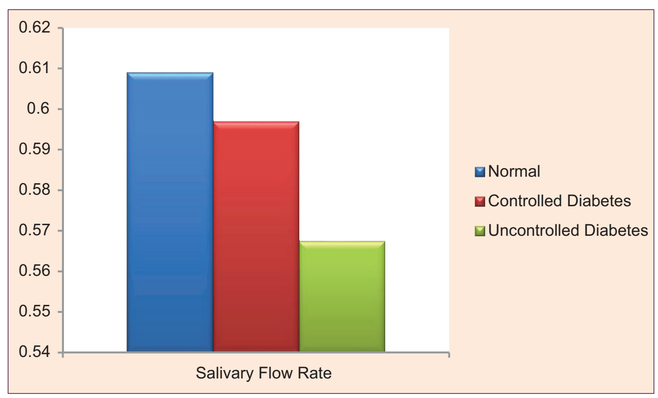

Figure 6: Salivary flow rate

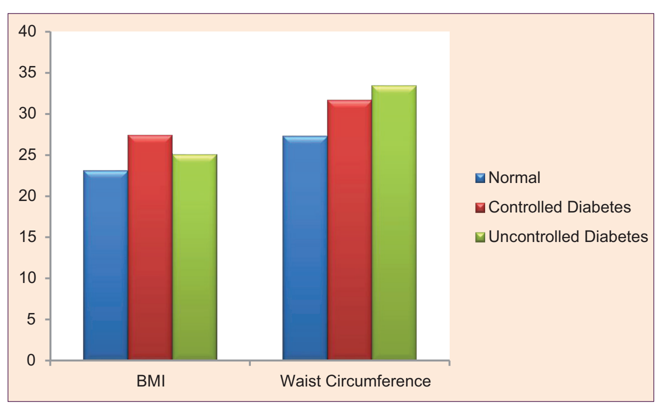

Figure 7: Comparison of body mass index and waist circumference in all groups

by Ben-Arey *et al.*, 1993, Ayadin, 2004 and Vasconcelous *et al.*, 2010, Agrawal *et al.*, 2013,[3] Jha *et al.*, 2014. However, the following studies did not show any correlation with our study , López *et al.*, 2003,[4] Carda *et al.*, 20065 and Hegde *et al.*, 20106 who found no correlation between glucose concentrations in blood and saliva in diabetic patients.[1]

Increased salivary glucose is attributed to the fact that glucose is a small molecule that easily diffuses through semi-permeable membranes. Thus, large amounts of glucose become available to saliva when blood glucose levels are elevated, as in diabetes.[7] Alterations in the permeability, occurring as a result of basement membrane changes in diabetes, may be an additional explanation for the increased concentration of glucose in saliva.[3,6] This alters the microvasculature structure and makes it more permeable. The end result is a leaky microvasculature and a leaky basement membrane, which explains the increased passage of glucose from the blood into the saliva in diabetes mellitus.[1,4,8] Little is known concerning the relationship between diabetes and salivary biochemical parameters and the effect of these changes on oral health. Hence, in this study, we tried to study the level of electrolytes in saliva of Type 2 diabetes mellitus.

Lasisi *et al.*,[2] states saliva contains large quantities of potassium and bicarbonate ions. The concentrations of both sodium and chloride ions are several times less in saliva than in plasma. In this study, we found no significant difference in salivary sodium of healthy and diabetic persons, and this was a similar finding in studies by Carda *et al.* 2006,[5] Kallapur *et al.*,[9] 2013, Shirzaiy *et al.*,[10] 2013. However, Hegde, *et al.*, 2014, proved that the concentration of sodium (Na), potassium (K) and -chloride (Cl) ions in saliva was higher in diabetic patients. Sharon *et al.*, 1985, in an animal study had proposed that in diabetes mellitus an autonomic neuropathy exists which causes sympathetic-parasympathetic imbalance. This imbalance may perhaps exert a continuous stimulation on the salivary glands, bringing about increased potassium secretion into the saliva.11 Yavuzyilmaz *et al.*,[11] 1996, Mata *et al.*, 2004[12] proved that the concentration of potassium can be reduced in diabetics when compared to normal persons, whereas, Andelski-Radicevic *et al.*, 2006, stated that the concentration of potassium in saliva of diabetic patients was higher than in healthy subjects, due either increased activity of Na K ATPas or due to the changes in the basal membrane of salivary gland acini or decrease in salivary secretion.[13] This study showed no significant difference between serum and salivary potassium levels. Diabetic patients have demonstrated an increase in calcium concentration in saliva compared to the control group according to Harrison *et al.*, 1984, Mata *et al.*, 2004. Busato *et al.*, 2011, it was considered that a high concentration of calcium in saliva is favorable indicator of oral health.[14] However, the results in this study were no significant correlation with this fact, revealing a decrease in salivary calcium concentration in controlled diabetics ($P = 0.01$) uncontrolled diabetics ($P = 0.05$ level). The decrease in salivary calcium in diabetics and may be attributed to increase in the of concentration of specific proteins special bonds with calcium phosphate and sometimes with hydration status of the individual. According to Lopez *et al.*, 2003 Iqbal *et al.*, 2011, Kumar 2012, Prathiba *et al.*, 2013. 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 appears in the range of 0.10-0.01 ml/min, with a decrease in flow rate.[15] This study showed no statistically significant difference between normal, diabetic and non-diabetic patients. This contradicted findings of Cherry-Peppers *et al.*, 1992, Karjalainen *et al.*, 1996, Lopez *et al.*, 2003, Prathiba *et al.*, 2013, who concluded that salivary flow rate was significantly diminished in diabetics as compared to that in non-diabetics due to excessive thirst and dry mouth, poor glycemic control increased diuresis and fluid loss. Several studies which were done on resting salivary pH estimated a range of 5.5-7.9 in normal individuals. The pH of saliva is maintained by carbonic acid and bicarbonate system, phosphate system and protein system of buffers. Prathiba *et al.*, 2013, Kennath, 2014 showed a significant decrease in pH in diabetics in comparison to non-diabetic subjects. This study, however, failed to demonstrate such a correlation between the diabetics and non-diabetics.

The WHO, 1998 reports indicated that the risk for diabetes increased three times in subjects with BMI more than 30 kg/m. The stronger association with weight gain in earlier adulthood than in later adulthood might be explained by the longer duration of exposure to cumulative excessive body fat. As postulated that the duration of obesity is a significant risk factor for type 2 diabetes, independently of the current degree of obesity, this study showed a positive correlation that showed increased BMI in uncontrolled diabetics.[16-18] This was in concordance with studies by Chan *et al.*, 1994, Willet *et al.*, (1999) and Anja Schienkiewitz *et al.*, 2006. Ishikawa-Takata, 2002[19] however proved that the risk for diabetes in subjects with BMI <29 kg/m^2 was not significant. There is a good indication of waist circumference being an important indicator of progression to diabetes than BMI. According to Williamson *et al.*, 2000, a variety of intervention studies show that patients with type 2 diabetes who succeed in losing weight often enjoy modest improvements in glycemic control and cardiovascular risk profilesm,[20] as long as the weight loss is maintained.[21] Pinkney, 2002, suggested that obesity is a major potentially modifiable risk factor for type 2 diabetes. This is similar to the association between obesity and diabetes shown in other studies in uncontrolled diabetes patients ($P = 0.000$) present study also showed a significant correlation between waist circumference and uncontrolled diabetes. Henry *et al.*, 2004, states that weight loss in overweight patients with type 2 diabetes rapidly reverses the state of insulin resistance and can restore normal blood glucose concentrations. Hence, there is a strong scope of waist circumference being an important indicator of progression to diabetes. This is due to a hypothesis that waist circumference relates to that, central obesity has been associated with decreased glucose tolerance, alterations in glucose-insulin homeostasis, reduced metabolic clearance of insulin, and decreased insulin-stimulated glucose disposal.[22]

CONCLUSION

This study along with many other studies indicates that saliva may be utilized as a non-invasive tool for evaluation of type 2 diabetes mellitus. Further research on larger samples with more clinical trials and in depth analysis for the imbalance of electrolytes which varies from one study to another should be done. This should be correlated with oral manifestations and other comorbidities to make saliva as a reliable tool.

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