Estimation and comparison of salivary flow rate and its composition in diabetic patients and nondiabetic patients: A pilot study

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INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia irregularities in the metabolism of carbohydrates, lipids and protein. It is often associated with the development of microvascular and macrovascular complications and neuropathies. The health of oral tissues is known to be related to the quality and quantity of saliva both of which may be altered in diabetes.

Aim: The aim of the present study was to determine the salivary flow rate, electrolytes and total proteins in saliva of Type II diabetic patients.

Materials and Methods: A total number of 120 participants were included in this study, in which 80 patients were suffering from Type II DM (which included both controlled and uncontrolled diabetes) and 40 nondiabetic persons (controls). The study population included both the genders, with an age range of 40–70 years. The study population was divided into three groups.

Results: The values of total protein, sodium, potassium and salivary flow rate among controls, controlled diabetes and uncontrolled diabetes were collected, formulated and multiple comparisons between the groups using the analysis of variance and post hoc Tukey honestly significant difference analysis were done in version 16.0 of SPSS software.

Conclusion: Studies with larger sample size are warranted to know the exact pathophysiology of controlled and uncontrolled Type II DM in terms of salivary flow rate, salivary electrolytes and total protein.

Keywords: Diabetes mellitus, potassium, saliva, salivary flow rate, sodium, total protein
and ulceration. Thus, diabetes is known as a complex disease with deleterious effects on the general health of an individual.

Various studies have established diabetes as a risk factor for the development of oral diseases in humans. It is probably the most common condition with salivary implication.

The health of oral tissues is known to be related to the quality and quantity of saliva both of which may be altered in diabetes. Several studies have been conducted to investigate salivary composition in participants with various systemic diseases. Conditions such as dental caries and periodontitis have been long identified as the recognizable features of DM. Furthermore, majority of patients with diabetes, complain of xerostomia (dry mouth) due to overall decrease in flow of saliva due to systemic dehydration and an increase in the salivary glucose level. Various underlying pathologies such as reduced salivary flow, delayed wound healing and atherosclerosis have been suggested to explain the increased prevalence of oral diseases in individuals with diabetes; however, the composition of saliva in these conditions needs further research.

Since diabetes is known to influence the salivary composition and function, the present study was carried out to estimate salivary flow rate, electrolytes and total proteins in Type 2 diabetes and to assess the correlation between the nondiabetic, controlled diabetic and uncontrolled diabetic patients using standard procedure. The aim of the present study was to determine the salivary flow rate, electrolytes and total proteins in saliva of Type II diabetic patients and use the results to follow-up and manage the diabetes for oral health issues.

MATERIALS AND METHODS

Patient selection
A total number of 120 participants were included in this study, in which 80 patients were suffering from Type II DM (which included both controlled and uncontrolled diabetes) and 40 nondiabetic persons (controls). Study population included both the genders, with an age range of 40–70 years.

The study population was divided into three groups.

Group I. (nondiabetes)
Group I comprised 40 patients 40–70 years of age with random nonfasting plasma glucose values ≥80 mg/dl and ≤120 mg/dl.

Group II: (controlled diabetes)
Group II comprised of 40 patients 40–70 years of age who were being treated for diabetes and had random nonfasting plasma glucose values >120 mg/dl and ≤200 mg/dl.

Group III: (uncontrolled diabetes)
Group III comprised of 40 patients 40–70 years of age who were being treated for diabetes and had random nonfasting plasma glucose values >200 mg/dl.

Inclusion criteria
• Patients having Type II diabetes
• Voluntary participation
• Sex: Both the genders.

Exclusion criteria
• Patients having other systemic diseases and on regular medication for the same
• Pregnant women
• Physically and mentally challenged persons.

Sample collection
All participants were explained in detail about this study and an informed consent was obtained in their native languages to prevent language bias and later was subjected to collection of saliva.

Saliva collection was undertaken between 10 and 11 a.m., and participants were instructed to have their breakfast not later than 8 a.m. Un-stimulated saliva was collected by the spitting method.

“Spit technique” was used for collection. The patient was made to sit in the chair with head tilted forward. They were instructed not to speak, swallow or do any head movements during the procedure. The patient was instructed to spit in a sterile graduated container every minute for 10 min.

Salivary flow rate was calculated for every patient by using the formula

\[
\text{Salivary Flow Rate} = \frac{\text{Post weight measure} - \text{Pre weight measure}}{\text{Collection period}} \quad \text{g/minute}
\]

Unstimulated saliva of 2 ml collected was used to evaluate electrolytes such as sodium, potassium and total proteins.

The testing of salivary samples was done in aseptic conditions. The unstimulated saliva of subjects was collected in a preweighed containers and immediately after collection, the bottles were examined to determine
the volume and stored at −20°C until used for laboratory analysis. Samples were defrosted at the room temperature and then centrifuged at 6000 rpm for 10 min before being used to remove contaminants such as oral epithelial cells, micro-organisms and food debris among others.

The specimens were analyzed in the room temperature and were fed into automated analyzer for interpretation of the following parameters:

Salivary ions analysis
The saliva collected was analyzed for the concentrations of potassium (K+), sodium (Na+). For the determination of salivary ions, saliva was diluted at either 1/100 or 1/1000 and K+, Na+ concentrations were determined using Roche 9180 electrolyte analyzer.

Salivary analysis of total protein
Saliva samples were defrosted at the room temperature and then centrifuged at 6000 rpm for 10 min before use. Total protein concentration expressed as mg/dl was determined using established automatic analyzer.

RESULTS
The values of total protein, sodium, potassium and salivary flow rate among controls, controlled diabetes and uncontrolled diabetes were collected, formulated and multiple comparisons between groups using analysis of variance and post hoc Tukey honestly significant difference analysis were done in Version 16.0 Statistical Package for the Social Sciences (SPSS), IBM Corporation, Chicago, United States of America.

The values of fasting blood sugar level in the Group 1 were in the range from 79 mg/dL to 96 mg/dL with an average of 88.9 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 1 in the range from 132 mEq/L to 149 mEq/L with an average of 139.05 mEq/L [Table 1 and Graph 2]. The values of potassium of the Group 1 were in the range from 3.4 mEq/L to 4.9 mEq/L with an average of 4.04 mEq/L [Table 1 and Graph 3]. The values of total protein of the Group 1 were in the range from 6.0 g/dL to 9.2 g/dL with an average of 7.28 g/dL [Table 1 and Graph 4]. The values of salivary flow rate in the Group 1 were in the range from 0.6 ml/min to 1.6 ml/min with an average of 1.09 ml/min [Table 1 and Graph 5].

The values of fasting blood sugar level in the Group 2 were in the range from 142 mg/dL to 178 mg/dL with an average of 160.35 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 2 were in the range from 146 mEq/L to 185 mEq/L with an average of 168.15 mEq/L [Table 1 and Graph 2]. The values of potassium of the Group 2 were in the range from 8.5 mEq/L to 10.6 mEq/L with an average of 9.45 mEq/L [Table 1 and Graph 3]. The values of total protein of the Group 2 were in the range from 5.2 g/dL to 8.2 g/dL with an average of 6.53 g/dL [Table 1 and Graph 4]. The values of salivary flow rate in the Group 2 were in the range from 0.4 ml/min to 1.0 ml/min with an average of 0.63 ml/min [Table 1 and Graph 5].
The values of fasting blood sugar level in Group 3 were in the range from 186 mg/dL to 303 mg/dL, with an average of 237 mg/dL [Table 1 and Graph 1]. The values of sodium of the Group 3 were in the range from 144 mEqL to 178 mEqL with an average of 156.3 mEqL [Table 1 and Graph 2]. The values of potassium of the Group 3 were in the range from 5.3 mEql to 6.9 mEqL with an average of 6.05 mEqL [Table 1 and Graph 3]. The values of total protein of Group 3 were in the range from 8.5 g/dl to 10.7 g/dl with an average of 9.3025 g/dl [Table 1 and Graph 4]. The values of salivary flow rate in the Group 3 were in the range from 0.4 ml/min to 0.7 ml/min with an average of 0.54 ml/min [Table 1 and Graph 5].

There was a distinct increase in values of total protein, sodium, potassium and decrease in salivary flow rate among the controlled diabetic and uncontrolled diabetic group. The values were found to be statistically significant ($P < 0.05$) [Table 2]. Within the groups of controlled and uncontrolled diabetes, there seemed to be
to compare them between the controlled and uncontrolled diabetic patients. The study population ($n = 120$) was divided into three groups, namely Group 1, i.e., healthy subject ($n = 40$), Group 2, i.e., controlled diabetes ($n = 40$) and Group 3, i.e., uncontrolled diabetes ($n = 40$). Saliva was collected from the sample population and was biochemically analyzed.

In this present study, the total protein level is increased in the diabetic patients (Group 3) when compared to nondiabetic participants. This is in agreement with Arati et al.\textsuperscript{11} and Streckfus et al.\textsuperscript{12} who demonstrated highly significant positive correlations in salivary total protein levels among uncontrolled and controlled diabetic groups. This could be attributed to the increase in basement membrane permeability, allowing easy and increased passage of serum proteins into the whole saliva through salivary gland and gingival crevices.

Mata et al.\textsuperscript{13} reported increased salivary protein concentration in diabetic patients, which was attributed to reduced salivary fluid secretion. This study is also in agreement with our findings where salivary flow rate is inversely proportional to the total protein level [Table 4, Graphs 4 and 5].

In this current study, we found statistically significant differences in salivary flow rate between controlled, uncontrolled diabetic group and healthy non diabetic group [Table 4 and Graph 5]. Salivary flow rate is decreased in diabetes patients when compared to the healthy participants [Graph 5].

The decrease in salivary flow rate occurring in diabetes can be factorial, 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. It can also be due to local inflammation and irritation in the oral cavity, metabolic disturbances and neuropathy affecting the salivary glands or as a result of drug therapy for diabetes and concomitant drugs.

The result of the study done by Meurman et al.\textsuperscript{14} contradicted with the finding of the present study as it showed no significant differences in the salivary flow rate. This may be attributed to the differences in sample selection and variation of environmental factors.

With respect to potassium, salivary concentration of this ion was found to be increased in diabetic patients when compared with nondiabetic individuals in the present study. Similar finding had been reported by Lasisi and Fasanmade,\textsuperscript{15} Mata et al.\textsuperscript{13}
Study done by Ben‑Aryeh et al.[16] is also in accordance with our findings. Elevation of potassium concentration in saliva of diabetic patients is probably secondary to diabetes induced decrease in salivary fluid output.[13] This might be due to intact secretory capacity of the salivary glands in Type 2 diabetes. In contrast, Streckfus et al.[12] and Marder et al.[17] documented that there is no difference in the potassium level in diabetic patients in their studies.

The salivary concentration of sodium was found to be increased in the diabetes group when compared to the controlled group the present study. This finding is in positive agreement with the study conducted by Basavaraj et al.[18] The reason could be due to decreased salivary flow rate which in turn increases the concentration of the sodium ion in saliva of diabetic patients.

In contrast, a study done by Lasisi and Fasanmade[15] found no significant difference in salivary sodium level in their diabetic patient’s sample.

On intergroup comparison in the present study, barring salivary flow rate and total protein level, electrolytes such as sodium and potassium showed statistically significant increase in controlled diabetics against the uncontrolled ones. This can be attributed to the following probable reasons:

- Smaller sample size
- Compromise of salivary flow in poorly controlled diabetes. This is in accordance with the study done by Rosamund and William[19] which in turn leads to altered salivary flow rate
- Effect of certain drugs taken by the study group volunteers for other underlying systemic diseases, which may not have been disclosed by them.

**CONCLUSION**

Thus, studies with larger sample size are warranted to know the exact pathophysiology of controlled and uncontrolled Type II DM in terms of salivary flow rate, salivary electrolytes and total protein.
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Conflicts of interest
There are no conflicts of interest.

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