Salivary flow and composition in diabetic and non-diabetic subjects

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Summary: The study investigated the effects of type 2 diabetes mellitus on salivary flow and composition in humans compared to healthy sex and age matched controls. Forty adult human subjects divided into 20 diabetic and 20 non-diabetic healthy subjects were included. Saliva samples were collected and analysed for glucose, total protein, calcium, sodium, potassium, chloride and bicarbonate. Salivary flow rate was also determined. The results showed that salivary glucose and potassium levels were significantly higher (p = 0.01 and 0.002 respectively) in diabetic patients compared with non-diabetic participants. It was also found that the diabetic patients had significant reduction in salivary flow rate when compared with non-diabetic individuals. In contrast, there was no significant difference in levels of total protein, Na^+, Ca^{++}, Cl^- and HCO_3^- between the two groups. These results suggest that some oral diseases associated with diabetes mellitus may be due to altered levels of salivary glucose, potassium and flow.

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INTRODUCTION

The understanding of the role of each salivary component in the oral cavity homeostasis is crucial to perceive how its changes or absence may be linked with pathological conditions (Mandel, 1993). The most commonly used laboratory diagnostic procedures involve the analyses of the cellular and chemical constituents of blood. Other biologic fluids are utilized for the diagnosis of disease, and saliva offers some distinctive advantages (Kaufman et al., 2002).

Biochemical analysis of saliva would be of great biomedical importance, since saliva is very easy to collect offering a cost-effective approach for screening of large populations, and could represent an alternative for the patient whose blood is difficult to obtain when compliance is a problem (Kaufman et al., 2002).

Diabetes is a widespread metabolic disease causing well-documented deleterious effects on the general health of an individual (Nechifor et al., 2001). Multiple epidemiologic studies have suggested that diabetes is a risk factor for the development of oral diseases in humans (Cianciola, 1982 and Manfredi et al., 2004). Diabetes is probably the most frequent metabolic disease with salivary implication (Mata et al., 2004). About a third of diabetic patients complain of dry mouth (Xerostomia) which may be due to overall diminished flow of saliva resulting from systemic dehydration and an increase in the salivary glucose level (Sreebny et al., 1992). Salivary glands hypo functions and increased susceptibility to oral infections such as caries or periodontitis (Twetman et al., 2002) have long been recognised features of diabetes mellitus, particularly when there has been dehydration and inadequate blood glucose control (Chavez et al., 2002).

A wide range of underlying pathogenic factors such as arteriosclerosis, reduced salivary flow rate and impaired wound healing has been postulated to explain the increased prevalence and severity of oral diseases observed in diabetes but the role of salivary composition in these diseases needs further studies.

There is little information on the effects of diabetes on salivary parameters and how these parameters may influence oral conditions.

This study was therefore designed to investigate the salivary flow and composition among diabetics and non-diabetic subjects.

MATERIALS AND METHODS

Study population
The study employed 40 human subjects (20 type ll diabetic patients and 20 healthy non-diabetic age and sex matched). The diabetic subjects were consecutive patients attending the Endocrine Unit of the Medical Out Patients Department, University College Hospital, Ibadan, while non-diabetic subjects were members of the University of Ibadan community.

The study received ethical clearance and approval by the University of Ibadan/University College Hospital health and research ethics committee Participants were provided information regarding risks and benefit of the study and consent was taken.

Saliva collection

Saliva collection was undertaken between 8am and 9am and participants were instructed to be in a fasting state (overnight fasting). Unstimulated saliva was collected by spitting method (Karin et al, 1999). Participants were asked to spit (after rinsing the mouth with deionized water) into calibrated universal plastic bottles for a period of 10 minutes. Rates of resting saliva secretions were expressed in mls/mins. Volumes of the secretions were recorded and stored at -20^0C for further analysis. Saliva samples were defrosted at room temperature and then centrifuged at 6000 rpm for 10 minutes before being used in order to remove extrinsic contamination elements such as oral epithelial cells, micro-organisms and food debris among others (Cimasoni, 1994, Cardan et al 2006).

Analysis of salivary ions

Saliva collected was analysed for the concentrations of K+, Na+, Ca2+, Cl- and HCO3^{2-}.

For the determination of salivary ions, saliva was diluted at either 1/100 or 1/1000 and K+, Na+ and Ca2+ concentrations were determined using flame emission spectrophotometry. Concentrations of Cl- and HCO3^{2-} were determined by Schales method using mercuric nitrate.

Analysis of salivary glucose

This was carried out by glucose oxidase method using 4-Aminophenazone as oxygen acceptor.

Analysis of salivary total protein

Saliva samples were defrosted at room temperature and then centrifuged at 6000 rpm for 10 minutes before use. Total protein concentration expressed as mg/dl was determined using established colorimetric methods with the use of Helios spectrophotometer by reading samples at 720nm.Bovine serum albumin was used for calibration purposes.

Statistical analysis

The main outcome variables were mean values of salivary flow rate, sodium, potassium, chloride, bicarbonate and total proteins in patients with type II diabetes and healthy control subjects. The data were initially explored using SPSS version 15 and variables were analysed using student t test. The level of statistical significance was set at $p < 0.05$.

RESULTS

The age and sex distribution of all subjects in the study are given in Table 1. The mean salivary levels

of calcium, sodium, potassium, chloride, bicarbonate and total proteins were 5.22 ± 1.19 mg/dl, 12.4 ± 7.97 mmol/L, 23.35 ± 5.61 mmol/L, 19.2 ± 3.21 mmol/L, 8 ± 2.77 mmol/L and 1.44 ± 0.82 mg/dl respectively in diabetic subjects; while the mean levels were 5.51 ± 1.74 mg/dl, 10.2 ± 6.45 mmol/L, 18.49 ± 4.79 mmol/L, 17.1 ± 3.58 mmol/L, 6.85 ± 2.56 mmol/L and 1.24 ± 0.98 mg/dl respectively in non-diabetic control subjects. There was no significant differences ($p > 0.05$) in salivary levels of these parameters except potassium and glucose in diabetic subjects when compared with non-diabetic control subjects.

Table 1. Demographic distribution of diabetic and non-diabetic subjects

Variable	Diabetic subjects	Non-diabetic subjects
Males	10	11
Females	10	9
M : F	1 : 1	1.2 : 1
Age ± SD (years)	58.4 ± 10.6	50.2 ± 9.2
Range	35 to 76	31 to 65
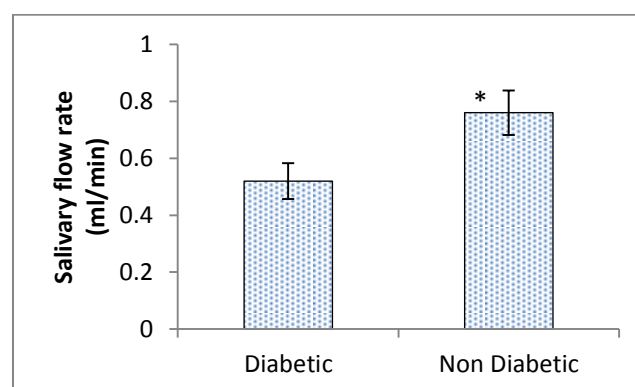
Figure 1. Salivary flow rate in the diabetic and the nondiabetic subjects, *P=0.04

Table 2: Mean salivary levels of glucose, electrolytes and total proteins in diabetic subjects and non-diabetic subjects

Variable	Diabetic subjects	Non-diabetic subjects
Calcium (mg/dl)	5.22 ± 1.19	5.51 ± 1.74
Sodium (mmol/L)	12.4 ± 7.97	10.2 ± 6.45
Potassium (mmol/L)	23.35 ± 5.61	18.49 ± 4.79*
Chloride (mmol/L)	19.2 ± 3.21	17.1 ±3.58
Bicarbonates (mmol/L)	8 ± 2.77	6.85 ± 2.56
Total protein (mg/dl)	1.44 ± 0.82	1.24 ± 0.98
Glucose (mg/dL)	106.1 ± 24.2	71.5 ± 1.9*

 * Significant at $p < 0.05$

Mean salivary potassium and salivary glucose levels were significantly elevated ($p = 0.01$ and 0.002 respectively) in diabetic subjects when compared with non-diabetic subjects (Figure 1 & Table 2). Salivary flow rate in diabetic individuals was significantly reduced (p=0.04) compared with non-diabetic controls (Figure 1).

DISCUSSION

In this study, the salivary glucose level of diabetic patients was found to be significantly higher than that of non-diabetic subjects, in consistent with previous findings (Ben - Arey et al., 1993, Ayadin, 2004 and Vasconcelous et al., 2010). The high salivary glucose level is a consequence of high plasma glucose level from which saliva is formed. This high salivary glucose in conjunction with overall diminished flow of saliva has also been reported to be responsible for the complaint of dry mouth by the diabetic patients (Twetman et al., 2002).

In addition, the high glucose level in saliva of diabetic patients might contribute to their susceptibility to oral infections like periodontal disease and dental caries (Tervonen & Kunnutila, 1986). The hyperglycemic environment can reduce tissue growth and matrix synthesis by fibroblasts and osteoclasts. As a result, the tissues are weaker and wound healing is delayed (Rammamuthy & Golub, 1983).

The results of this study showed a significantly reduced salivary flow rates in diabetic patients when compared with non-diabetic individuals. A similar finding was also reported by Meurman et al. (1998) and Dodds & Dodd (1997) suggesting the presence of diabetes - induced impairment of salivary gland function. Similar findings have also been previously described in the literature and were associated with diabetes induced-neuropathic changes in the salivary parenchyma with Iymphocythic gland infiltrate similar to the one occurring in the pancreas of these diabetic patients (Markopoulos & Belaxi, 1998). The reduced salivary flow rates have been reported to be more frequent in uncontrolled diabetic patients (Ship et al., 2002).

In this study, the salivary total protein level in diabetic patients was not significantly different from that of non-diabetic individuals. This is consistent with the findings of Dodds & Dodds (1997), although some studies reported increased salivary protein concentration in diabetic patients, which was attributed to reduced salivary fluid secretion (Antonio et al, 2004). The increase in salivary total protein level reported by some authors was more marked in type 1 diabetic patient when compared with type 2 which was the status of diabetic subjects used in this study. In addition, despite the fact that increased protein concentration in diabetic patients saliva has been described before by some authors (Twetman et al., 2002), there are also reports of diminished epidermal growth factor, antioxidant capacity and salivary oxidase activity suggesting that while some proteins may experience an enhanced output, others may be diminished (Beke et al., 2000, Oxford et al, 2000).

With respect to potassium, salivary concentration of this ion was found to be increased in diabetic patients when compared with non-diabetic individuals. Similar finding has been reported by Mata et al. (2004) and Yavuzyitmazet al., (1996). Elevation of potassium concentration in saliva of diabetic patients is probably secondary to diabetes induced decrease in salivary fluid output (Mata et al., 2004).

The present study showed no significant difference in salivary concentrations of calcium, sodium, chloride and bicarbonate in saliva of diabetic patients compared with non-diabetic controls. This is consistent with the report of Dodds et al. (2000) and Mata et al., (2004). This might be due to intact secretory capacity of the salivary glands in type 2 diabetes. Similarly, Mata et al. (2004) found no statistical difference for Ca^{2+} secretary capacity for the type II diabetes compared with non-diabetic individuals. In addition, Na^+, Cl^- and bicarbonate are secreted in minute concentrations in the saliva (Karin et al., 1999) and this might account for the absence of significant difference.

In conclusion, this study has shown that the salivary composition in diabetics and non-diabetics are almost similar except for potassium and glucose which are higher in diabetic patients and these may be contributing factors to oral diseases.

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