Ann Ibd. Pg. Med 2012. Vol.10, No.1 25-30

COMPARATIVE ANALYSIS OF SALIVARY GLUCOSE AND ELECTROLYTES IN DIABETIC INDIVIDUALS WITH PERIODONTITIS

T.J. Lasisi[1] and A.A. Fasanmade[2]

1. Department of Physiology, University of Ibadan, Ibadan
2. Department of Medicine, Endocrinology Unit, University College Hospital, Ibadan

Correspondence
Dr. Taye J. Lasisi
Department of Physiology,
University of Ibadan, Ibadan
E-mail: jameelahlasisi@yahoo.com

ABSTRACT

***Background*: A high incidence of periodontal disease has been reported among diabetics, however the role of saliva in the occurrence of this oral disease in these patients is yet to be understood.**

***Objective:* To determine the effects of type-2 diabetes and periodontal disease on salivary flow rate and biochemical composition.**

***Design:* A prospective study involving 40 adult human subjects divided equally into four groups of diabetics with periodontitis (group 1), diabetics without periodontitis (group 2), non diabetics with periodontitis (group 3) and non diabetics without periodontitis (group 4).**

***Methodology:* Saliva samples were collected and analyzed for salivary glucose, total protein, calcium, sodium, potassium, chloride and bicarbonate. Salivary flow rates were also determined.**

***Results:* Salivary glucose and potassium levels were significantly higher (P = 0.002 and 0.04 respectively) in diabetic patients regardless of periodontal disease (mean = 100.7 ± 9.33 mg/dl; 111.5 ± 32.85 mg/dl and 23.79 ± 5.19 mg/dl; 22.9 ± 6.25 mg/dl respectively) compared with non diabetic participants (mean = 80.5 ± 30.85 mg/dl; 62.5 ± 31.89 mg/dl and 19.23 ± 5.04 mg/dl; 17.74 ± 4.68 mg/dl respectively). In contrast, there was no significant difference in saliva flow rates and levels of total protein, Na^+, Ca^{++}, Cl^- and HCO_3^- between the groups.**

***Conclusion:* Salivary glucose and potassium levels were significantly higher among diabetics with or without periodontitis compared with non-diabetics with or without periodontitis. However, biochemical composition of saliva in diabetic individuals has probably little role in their susceptibility to periodontitis.**

Keywords: periodontitis, type 2 diabetes mellitus, salivary electrolytes, salivary glucose

INTRODUCTION

The role of saliva in oral health has been a subject of continued research[1,2]. However, the study of salivary functions has been challenging because of the high physiological variability of this fluid when compared to other body fluids such as plasma[3,4].

Several systemic diseases affect oral health and diabetes mellitus is an established risk factor for periodontitis.[5] Most studies reported that periodontitis and xerostomia are common complications of both insulin dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM)[6,7,8]. These oral complications have been attributed to diminished flow of saliva resulting from systemic dehydration and an increase in the salivary glucose level[8-10]. A wide range of underlying pathogenic factors has been postulated to explain the increased prevalence and severity of periodontitis[11,12] observed in diabetes but the role of salivary composition has not been fully studied. Furthermore, little is known concerning the relationship between diabetes and salivary biochemical parameters and the effect of these changes on oral health. In addition, despite the fact that periodontitis is highly prevalent among diabetics, most studies do not relate the salivary composition in diabetic patients to periodontitis.

The aim of this study was to determine the effects of diabetes and periodontal disease on salivary flow rate and biochemical composition.

METHODOLOGY

Study Design

This is a prospective study involving 40 adult human subjects divided equally into four groups of diabetic with periodontitis (group 1), diabetic without periodontitis (group 2), non diabetic with periodontitis (group 3) and non diabetic without periodontitis (group 4).

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.

Participants were provided information regarding risks and benefit of the study and consents were taken. Diagnosis of periodontal disease was based on findings using the attachment loss scoring system (CPI Index). Subjects with periodontal indices of ≥ 3 were considered as having periodontitis while those with periodontal indices of ≤ 2 were considered as not having periodontitis.

The study received ethical clearance and approval from the Joint University of Ibadan/University College Hospital Health Research Ethical Committee.

Saliva collection

Saliva collection was undertaken between 8am and 9am and participants were instructed to observe overnight fast (last meal not later than 12 midnight). Un-stimulated saliva was collected by the spitting method[13]. 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.

Immediately after collection, the bottles were examined to determine the volume and stored at -20^{0}C until used for laboratory analysis. Samples were defrosted at room temperature and then centrifuged at 6000 rpm for 10 minutes before being used in order to remove contaminants such as oral epithelial cells, micro organisms and food debris among others.

Salivary ions analysis

The saliva collected was analyzed for the concentrations of K^+, Na^+, Ca^{2+}, Cl^- and HCO_3^{-2}.

For the determination of salivary ions, saliva was diluted at either 1/100 or 1/1000 and K^+, Na^+ and Ca^{2+} concentrations were determined using flame emission spectrophotometry[14]. Concentrations of Cl^- and HCO_3^{2-} were determined by Schale's method using mercuric nitrate[15].

Salivary glucose analysis

This was carried out by the glucose oxidase method using 4-Aminophenazone as oxygen acceptor[16].

Salivary analysis of 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[17].

Statistical analysis

All statistical analyses were carried out using SPSS Version 16. The Analysis of Variance (ANOVA) and Duncan tests were employed to evaluate differences within and between the groups. A p-value of 5% was considered to be statistically significant.

RESULTS

Forty subjects were recruited into the study consisting of 21 males and 19 females with a mean age of 54.3

	Group 1	*Group 2*	*Group 3*	*Group 4*
Males	5	5	7	4
Females	5	5	3	6
Age in years (range, mean ± SD)	52 to 76, 62.6 ± 9.1 yrs	35 to 65, 54.2 ± 10.7 yrs	45 to 60, 55.3 ± 7.0 yrs	31 to 52, 45.1 ± 8.5 yrs
Periodontal index (mean ± SD)	4.1 ± 0.7	1.9 ± 0.3	3.7 ± 0.5	1.8 ± 0.4

Group 1: Diabetics with periodontitis, Group 2: Diabetics without periodontitis, Group 3: Non-diabetics with periodontitis, Group 4: Non diabetics without periodontitis.

Table 1: Demographic distribution of subjects

Group	Glucose Mean ± SD (mg/dl)	Potassium Mean ± SD (mmol/L)
1	100.7 ± 9.3	23.9 ± 5.2
2	111.5 ± 32.9	22.9 ± 6.3
3	80.5 ± 30.9	19.2 ± 5.1
4	62.5 ± 31.9	17.7 ± 4.7
p value	0.002	0.04

Group 1: Diabetic with periodontitis, Group 2: Diabetic without periodontitis,
Group 3: Non-diabetic with periodontitis, Group 4: Non diabetic without periodontitis.

Table 2: Salivary glucose and potassium levels in diabetic patients with periodontitis (Group 1) and controls (groups 2, 3 and 4)

± 8.9 years. Table 1 shows the age, gender distribution and periodontal indices of the subjects. The subjects and controls were matched for age and gender (using ANOVA and chi square, p = 0.06 and 0.75 respectively). Among the groups, the mean ± SD of the salivary glucose levels were 100.7 ± 9.3 mg/dl, 111.5 ± 32.9 mg/dl, 80.5 ± 30.9mg/dl and 62.5 ± 31.9 mg/dl respectively while the salivary potassium levels were 23.9 mmol/L ± 5.2 mmol/L, 22.9 ± 6.3 mmol/L, 19.2 ± 5.1 mmol/L and 17.7 ± 4.7 mmol/L respectively. Salivary glucose and potassium levels were significantly higher among diabetics compared with non-diabetic groups irrespective of periodontal disease (p = 0.002 and p = 0.04 respectively) as shown in Table 2.

Table 3 shows the salivary levels of total protein, sodium, calcium, chloride and bicarbonate among diabetic patients with periodontitis and controls. There were no significant differences in the salivary levels of these substances between diabetic patients with periodontitis and controls (Table 3).

The salivary flow rates were reduced in diabetic patients regardless of periodontal disease with mean values of 0.6 ± 0.4 ml/min, 0.5 ± 0.2 ml/min, 0.9 ± 0.5 ml/min and 0.7 ± 0.2 ml/min respectively, although this was not statistically different when compared with non diabetic groups (p = 0.06) (Table 4).

Group	Total protein Mean ± SD (mg/dl)	Sodium Mean ± SD (mmol/L)	Calcium Mean ± SD (mg/dl)	Chloride Mean ± SD (mmol/L)	Bicarbonate Mean ± SD (mmol/L)
1	1.4 ± 0.8	10.6 ± 4.4	5.5 ± 1.5	19.2 ± 2.2	8.3 ± 3.1
2	1. ± 0.9	14.2 ± 10.4	4.9 ± 0.7	19.2 ± 4.1	7.7 ± 2.5
3	1.1 ± 0.8	10.8 ±8.0	4.8 ± 1.4	16.6 ± 4.6	7.1 ± 2.4
4	1.1 ± 0.8	9.6 ± 4.9	6.2 ± 1.8	17.6 ± 2.3	0.7 ± 0.2
p value	0.80	0.53	0.11	0.27	0.54

Group 1: Diabetic with periodontitis, Group 2: Diabetic without periodontitis,
Group 3: Non-diabetic with periodontitis, Group 4: Non diabetic without periodontitis.

Table 3: Salivary total protein, sodium, calcium, chloride and bicarbonate levels in diabetic patients with periodontitis (group 1) and controls (groups 2, 3and 4)

Group	Flow rate (ml/min)
1	0.6 ± 0.4
2	0.5 ± 0.2
3	0.9 ± 0.5
4	0.7 ± 0.2
p value	0.06

Group 1: Diabetic with periodontitis,
Group 2: Diabetic without periodontitis,
Group 3: Non-diabetic with periodontitis,
Group 4: Non diabetic without periodontitis.

Table 4: Salivary flow rates in diabetic patients with periodontitis (group 1) and controls (groups 2, 3 and 4)

DISCUSSION

In this study 40 adult human subjects were used. Age and sex matched non diabetic individuals were used as controls for the diabetic individuals and unstimulated whole saliva samples were collected from the subjects (in fasting states) similar to the work of Cardan *et al.*[18].

Salivary Glucose levels

In this study, the salivary glucose level of diabetic patients was found to be significantly higher compared with non diabetic subjects irrespective of periodontal disease. This is consistent with the findings of Aren *et al.*[19], Ayadin[20] and Vasconcelous *et al.*[21]. This result is an indication of plasma glucose level from which saliva is formed. This has also been reported to be responsible for the complaint of dry mouth by the diabetic patients due to overall diminished flow of saliva as a consequence of dehydration and an increase in the salivary glucose level[8]. In addition, the high glucose level in saliva of diabetic patients might contribute to their susceptibility to oral infections like candidiasis and dental caries and not necessarily periodontal disease.

Salivary Flow Rate

The results of this study showed no significant difference between the salivary flow rates of diabetic patients with periodontitis compared with controls. This finding was also reported by Meurman *et al.*[22] and Dodds *et al.*[23]. However, the Duncan test showed significant lower flow rate comparing diabetics with or without periodontitis with non-diabetics without periodontitis. This result suggests the presence of diabetes induced impairment of salivary gland function. Similar findings have been described in the literature and may be associated with diabetes induced-neuropathy changes in the salivary parenchyma with lymphocythic gland infiltrate similar to the one occurring in the pancreas of these patients[24]. These findings have been reported to be more frequent in uncontrolled diabetic patients[25]. However in this study, patients were selected from the outpatient diabetic individuals who were probably well controlled patients. This might account for the absence of significant difference in the salivary flow rate of diabetics compared with normal healthy controls (non diabetic subjects without periodontal disease).

Salivary Total Protein Levels

In the study, the salivary total protein level in diabetic patients with periodontitis was not significantly different compared with non diabetic individuals with or without periodontal disease. This is consistent with the findings of Dodds *et al.*[23] and Panchbai *et al.*[26] Although, some studies reported increased salivary protein concentration in diabetic patients, which was attributed to reduced salivary fluid secretion[1]. Again this increase was more marked in type 1 diabetic patient when compared with type 2, which was the status of diabetic subjects used in this study. Despite the fact that increased protein concentration in the saliva of diabetic patients has been described before by other authors[9], there are also reports of diminished epidermal growth factor[27], antioxidant capacity and salivary oxidase activity suggesting that while some proteins may experience an enhanced output, others may be diminished[28].

Salivary Potassium Levels

With respect to potassium, salivary concentration of this ion was found to be increased in diabetic patients with periodontitis compared with non diabetic individuals. The elevation was also higher in diabetic patients with periodontitis than those without periodontitis. This finding is consistent with that of Mata *et al.*[1], which reported significantly elevated salivary potassium concentration in diabetic patients. Elevation of potassium concentration in saliva of diabetic patients is probably secondary to diabetes induced decrease in salivary fluid output.

Salivary Ca^{2+}, Na^{+}, Cl^{-} and HCO_3 Levels

The study showed no significant difference in salivary concentrations of calcium, sodium, chloride and bicarbonate in saliva of diabetic patients with periodontitis compared with controls. This finding is similar to that of Ben-Ahrey[18] and might be due to intact secretary capacity of the salivary glands in type 2 diabetes. In addition, Na^{+}, Cl^{-} and bicarbonate are secreted in minute concentrations in the saliva[18] and this might account for the absence of significant difference.

CONCLUSION

Salivary glucose and potassium levels were significantly elevated in diabetics individuals irrespective of periodontal disease compared with non diabetics while

other parameters remain unchanged. However, this study has demonstrated that inorganic composition of saliva has little contributory role in the association of periodontal disease with diabetes and biochemical findings in saliva of diabetic patients is probably not very helpful in understanding the increased severity of periodontal disease in these individuals.

REFERENCES

1. **Mata AD**, Marques D, Rocha S *et al.* Effects of diabetes mellitus on salivary secretion and its composition in human. *Mol cell Biochem* 2004; 2: 137-142.
2. **Mandel ID.** Salivary diagnosis: more than a lick and a promise. *JAM Dent Assoc* 1993; 2:85-87.
3. **Fox PC**. Salivary monitoring in Oral diseases. *Ann NY Acad Sci* 1993; 694:234-237.
4. **Kaufman E,** Lamster IB. The diagnostic applications of saliva – a review. *Crit Rev. Oral biol Med* 2002; 13(2):197-212.
5. **Manfredi M,** McCullough MJ, Vescovi P *et al.* Update on diabetes mellitus and related oral diseases. *Oral Dis* 2004; 10:187-200.
6. **Cianciola LJ**, Park BH, Bruck E *et al.* Prevalence of periodontal disease in insulin dependent diabetes mellitus. *JAM Dent Assoc* 1982; 104: 653-660.
7. **Thorstensson H,** Falk H, Hugoson A, Olsso J. Some salivary factors in insulin dependent diabetes. *Acta odontologica scandinavica* 1989; 47:175-183.
8. **Sreebny LM**, Yu A, Green A, Valdini A, (1992). Xerostomia in diabetes Mellitus. *Diabetes care* 1992; 15(7):900-904.
9. **Twetman S**, Johansson I, Birkhed D, Nederfors T. Caries incidence patients in relation to metabolic control and caries associated risk factors. *Caries Res* 2002; 36(1): 312-335.
10. **Chavez EM**, Borrell LN, Taylor GW, Ship JA. Salivary function and glycemic control in older persons with diabetes. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; 89: 305-311.
11. **Steward JE**, Wager KA, Friedlander AH *et al.* The effect of periodontal treatment on glycemic control in patients with type 2 diabetes mellitus. *J Clin Perio* 2001; 4: 306-308.
12. **Giardino I,** Edelstein D, Brownlee M. Non-enzymic glycosylation in vitro and in bovine endothelial cells alters fibroblast growth factor. A model for intracellular glycosylation in diabetes. *J Clinical Invest* 1994; 94: 110-117
13. **Kaufman E,** Lamster IB. The diagnostic applications of saliva – a review. *Crit Rev Oral Biol Med* 2002; 13(2):197-212.
14. **Ferro PV**, Itam AB. A simple spectrophotometric method for determination of calcium. A*m J Clin Pathol* 1957; 28:208-217.
15. **Shannon IR,** John PR. Physiologic chloride levels in human whole saliva. *Exp Biol Med* 1958; 97:825-828.
16. **Sashikumar R**, Kannan R. Salivary glucose levels and oral candidal carriage in type II diabetics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; 109:706-711.
17. **Gornall AG**, Bardawill CJ, David MM. Determination of serum proteins by means of the Biuret reaction. *J Biol Chem* 1949; 177:751-752.
18. **Cardan C**, Mosqueva-Lloreda N, Salom L *et al.* Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Pathol Oral Cir Buccal.* 2006; 11: E309-314.
19. **Aren G.** Sepet E, Ozdemir D *et al.* Periodontal health, salivary status, and metabolic control in children with type 1 diabetes mellitus. *J Periodontol* 2003; 74(12):1789-1795.
20. **Ayadin SA**. Comparison of ghrelin, glucose, alpha amylase and protein levels in saliva from diabetics. *J Biochem Molecular Biol* 2007; 40: 29-35.
21. **Vasconcelos AC**, Soares MM, Almeida PC, Soares TC. Comparative study of the concentration of salivary and blood glucose in type 2 diabetic patients. *J Oral Sci* 2010; 52:293-298.
22. **Meurman JH,** Collin Hl, Niskanen L *et al.* Saliva in non-insulin dependent diabetic patients and control subjects; the role of autonomic nervous system. *Oral Surg Oral Med Oral Pathol, Oral Radiol Endod* 1998; 86:69-76.
23. **Dodds MW,** Dodds AP. Effects of *glycemic* control on saliva flow rates and protein composition in non – insulin dependent diabetes mellitus. *Oral Surg Oral Med Oral Pathol Oral Radiol Endol* 1997; 83(4): 465-470.
24. **Markopoulos AK,** Belaxi M. Histopathological and immunohistochemical features of labial salivary glands in children with type 1 diabetes. *J Diabetes Complications* 1998; 12:39-42.
25. **Ship JA,** Pillemer SR, Baum BJ. Xerostomia and the geriatric patient. *J Am Geriatr Soc* 2002; 50(3):535-543.

26. **Panchbai AS**, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Science 2010; 52: 359-368.
27. **Oxford GE,** Tayari L, Barfoor MD *et al.* Salivary EGF levels reduced in diabetic patients. J Diabetes Complications 2000; 14:140-145.
28. **Belce A**, Uslu E, Kucur M, *et al.* Evaluation of salivary sialic acid level and Cu-Zn superoxide dismutase activity in type 1 diabetes mellitus. J Exp Med 2000; 192(3): 219-225.
29. Ben Aryeh H, Serouya R. Kanter Y *et al.* Oral health and salivary composition in diabetic patients. J Diabetes Complication 1993; 7(1):57-62.