Study of the Buffering Capacity, pH and Salivary Flow Rate in Type 2 Well-Controlled and Poorly Controlled Diabetic Patients

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Purpose: This study measured the flow rate, pH and buffering capacity of saliva from well- and poorly metabolically controlled Type 2 diabetic patients in three cities of the southern part of Brazil, compared with healthy individuals from the same cities.

Materials and Methods: Whole saliva was collected by mechanical stimulation and buffering capacity and glucose level were measured. Blood was collected after 12 hours fasting and glucose and glycosylated haemoglobin concentrations were determined. The data were analysed by one-way ANOVA and Student-Newman-Keuls ($\alpha = 0.05$).

Results: The flow rate was lower in the Type 2 diabetic patients, regardless of whether they were well or poorly metabolically controlled, compared with healthy individuals ($p < 0.05$). Salivary glucose concentration was higher in both diabetic patient groups, i.e. well and poorly metabolically controlled, than in the control ($p < 0.05$).

Conclusion: The metabolic control of hyperglycaemia was not sufficient to improve the salivary flow rate or the salivary glucose concentration.

Key words: buffering capacity, flow rate, pH, saliva, Type 2 diabetes

Oral Health Prev Dent 2007;5: 73-78. *Submitted for publication: 14.12.05; accepted for publication: 27.04.06.*

According to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, diabetes mellitus is a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Diabetes includes two forms: Type 1 diabetes, in which the pancreas β-cells lose their capacity to produce insulin; and Type 2 diabetes, in which a defect in the β-cells or a reduction in tissue sensitivity to insulin are necessary for disease manifestation. Oral health complications associated with diabetes include xerostomia, tooth loss, gingivitis, and soft tissue lesions of the tongue and oral mucosa (Darnell and Saunders, 1990; Lamey et al, 1992; Loe et al, 1993).

Reports demonstrating high prevalence of dental caries in diabetic patients (Albrecht et al, 1988; Jones et al, 1992; Swanljung et al, 1992; Karjalainen et al, 1997; Twetman et al, 2002) are contradicted by reports showing no difference (Bacic et al, 1989; Edblad et al, 2001) or a lower prevalence of dental caries in diabetics than in healthy individuals (Matsson and Koch, 1975; Tavares et al, 1991).

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Salivary secretions are important for oral health, accomplishing mechanical cleansing and protective functions through a number of physiological and biochemical mechanisms. Saliva has protective actions by maintaining the integrity of oral mucous membrane by lubrication and soft tissue repair.

Salivary parameters, such as flow rate, pH, buffer capacity and saliva glucose concentration, have been evaluated in diabetic individuals, however, with conflicting results (Marder et al, 1975; Sharon et al, 1985; Lamey et al, 1986; Tenovuo et al, 1986; Chauncey et al, 1987; Ben-Aryeh, 1988; Thorstensson et al, 1989; Streckfus et al, 1994; Karjalainen et al, 1996; Belazi et al, 1998; Collin et al, 1998; Dodds et al, 2000; Chávez et al, 2001). Methodological differences including method of choice, sample selection approach, type of medication, patients with prosthesis, mouth breather patients, age and the diabetic status are factors that may have contributed to the conflicting results found in saliva of diabetics. Also, most of the information regarding saliva flow and diabetes is about the Type 1 form and therefore more studies should be conducted on the Type 2 form.

The aim of this investigation was to examine some salivary parameters as pH, flow rate, saliva glucose level, buffer capacity and self-report of xerostomia in stimulated saliva from Type 2 diabetic patients classified as well- and poorly metabolically controlled according to the glycosylated haemoglobin levels.

MATERIALS AND METHODS

Subjects

The study included 82 Type 2 diabetic individuals (31–86 years old) from the cities of Joaçaba, Ouro and Capinzal, located in the State of Santa Catarina, Brazil, with medically confirmed diagnoses and identified by WHO criteria, i.e. fasting blood glucose greater than or equal to 126 mg/dl. Those using total prostheses and mouth breathers were excluded from this study.

The diabetic patients were classified in two groups: (a) well controlled (WC), when the glycosylated haemoglobin (HbA1c) values were not greater than 8%; and (b) poorly controlled (PC), with HbA1c values greater than 8% (Matsson and Cerutis, 2001). The control group consisted of 18 subjects from the same cities, with no history of diabetes and a fasting blood glucose level lower than 110 mg/dl, and of similar age. The Ethics Committee from the University of Oeste of Santa Catarina approved the protocol of this study.

Assessment of self-report of xerostomia

The subjective experience of dry mouth (xerostomia) was assessed by asking the subjects the question, 'Does your mouth usually feel dry?' at the time of saliva collection.

Saliva collection

Stimulated whole saliva was collected in the morning (8–10 a.m.) to minimise the effect of circadian rhythms, in a well-ventilated and lighted room. The subjects were requested not to drink, eat or perform any physical activity for at least 2 hours before saliva collection. Whole saliva was collected for exactly 5 minutes, with mechanical stimulation, by chewing a base chewing gum (approximately 1 g). The saliva secreted in the first 30 seconds was degluted or discarded, after which 5 minutes were counted.

Blood collection

Venous blood from the arm was collected in the morning, after a 12-hour fasting period, by aspiration with a syringe, in the Laboratory of Clinical Analysis by a nurse.

Analysis

Blood and saliva glucose levels were measured by the glucose-oxidase method, using a kit (Labtest Diagnóstica, Brazil). Glycosylated haemoglobin was determined using the glycosylated haemoglobin kit (Labtest Diagnóstica, Brazil). Saliva pH was measured using a pH-meter (pHTEK, pH-100, São Paulo, Brazil) and the buffer capacity was measured by mixing 1 ml of saliva and 3 ml of 0.005 NHCl. After 5 minutes the final pH was measured.

Statistical analysis

The results of subjective experience of xerostomia from the three groups were compared using Fisher's exact test ($\alpha = 0.05$).

The results of saliva pH, flow rate, buffer capacity and saliva glucose levels were compared between three groups (poorly and well-metabolically controlled group and control) according to one-way ANOVA and Student-Newman-Keuls test ($\alpha = 0.05$). The correla-

Table 1 Demographic characteristics of Type 2 diabetics, including those with good (Hb A 1c < 8) and poor (Hb A 1c > 8) metabolic control

	PC	WC	Control
Age group (years)			
31–40	5	1	3
41–50	6	3	4
51–60	14	1	4
61–70	22	7	3
71–80	16	7	4
Total	63	19	18
Mean age ± SD	54.3 ± 10.1	63.6 ± 12.3	57.7 ± 15.6
Sex			
Female	38	11	13
Male	25	8	5
Medicated subjects (%)	82.5	89.5	0
Cigarette/alcohol users (%)	15.9	31.5	16.6
Blood glucose level (mean ± SD) (mg/dl)	169.0 ± 59.5	99.9 ± 11.2	91.3 ± 22.4
Hb A1c ± SD (%)	10.5 ± 1.8	7.2 ± 0.6	6.2 ± 0.15

PC, poorly metabolically controlled patients.
WC, well metabolically controlled patients.

Table 2 Number of individuals relating the feeling of dry mouth

Dry mouth	PC	WC	Control
	n (%)	n (%)	n (%)
Yes	34 (54)	9 (47)	0 (0)
No	29 (46)	10 (53)	18 (100)
Statistical analysis	a	a	b

PC, poorly metabolically controlled patients.
WC, well metabolically controlled patients.
a,b Same letter indicates means not statistically different. Different letter indicates means statistically different.

tions between saliva pH, flow rate, buffer capacity and saliva glucose levels and blood glucose levels were tested, as well as the correlations with glycosylated haemoglobin. The strength of the association between these variables was estimated with the Pearson product-moment correlation statistics ($\alpha = 0.05$).

RESULTS

The demographic characteristics of the participants in this study are shown in Table 1. Out of the 82 diabetic patients, 19 (23%) presented WC, by the criteria of glycosylated haemoglobin (Hb A1c < 8) and blood glucose levels (< 110.0 mg/dl), while 63 (77%) were classified as PC based on the same criteria of glycosylated haemoglobin (Hb A1c > 8) and blood glucose levels (> 126.0 mg/dl).

Table 2 shows the percentage of individuals that reported a feeling of dry mouth. No participant of the control group related a feeling of dry mouth. However, 54% and 47% of the patients from the PC and WC groups reported the feeling of dry mouth respectively ($p = 0.89$). No significant difference was observed between WC and PC groups ($p > 0.05$), which were both statistically different from the control group ($p = 0.0001$).

Table 3 Results of stimulated salivary flow rate, salivary glucose level, initial and final pH of the buffer capacity determination in each group

	PC	WC	Control	p-value
Flow rate (ml/min)	0.65 ± 0.62[a]	0.81 ± 0.47[a]	1.95 ± 0.73[b]	< 0.001
Saliva pH	6.8 ± 0.9[c]	6.7 ± 1.3[c]	6.7 ± 1.8[c]	0.309
Glucose (mg/dl)	12.5 ± 7.7[d]	12.7 ± 6.1[d]	3.7 ± 3.7[e]	0.001
Buffer capacity final pH	4.34 ± 1.58[f]	4.81 ± 1.21[f]	4.45 ± 1.45[f]	0.306

PC, poorly metabolically controlled patients.
WC, well metabolically controlled patients.
[a–f] Same letter in each row indicates means not statistically different. Different letter indicates means statistically different.

The flow rates, pH and buffer capacities of saliva obtained in the present study are shown in Table 3. The flow rates from the WC and PC were lower than that from the healthy group. While the PC group showed a mean flow rate 67% lower than the control subjects, a reduction of 58% was observed in the WC patients. With regard to salivary glucose levels, both diabetic groups showed glucose levels higher than the control group. No significant difference was observed in the pH after acid challenge (buffer capacity).

Fig 1 shows the dispersion diagram of the variables flow rate and blood glucose level and Fig 2 shows the dispersion diagram of the variables flow rate and glycosylated haemoglobin. In both figures it can be seen that there are negative linear correlations between the salivary flow rate and blood glucose ($r = -0.57$; $p = 0.0005$), and the salivary flow rate and glycosylated haemoglobin ($r = -0.61$; $p = 0.045$) concentrations.

DISCUSSION

Diabetes is a chronic disease with long-term duration, and an adequate metabolic control may delay the onset of the various health complications of the disease. In the present study, some parameters of stimulated whole saliva from patients with Type 2 diabetes (classified as WC and PC according to the glycosylated haemoglobin level) were studied, and compared with healthy individuals.

Changes in salivary flow rate in diabetic patients have been reported in several studies, with contradictory results. Reduced salivary flow rates in diabetics has been reported in some studies (Harrison and Bowen, 1987; Ben-Aryeh et al, 1988; Thorstensson et al, 1989; Newrick et al, 1991; Chávez et al, 2001), but not in others (Sharon et al, 1985; Lamey et alm 1986; Tenovuo et al, 1986; Dodds and Dodds, 1987; Streckfus et al, 1994).

In the present study, the stimulated salivary flow rates of the diabetic groups were 53% (WC group) to 67% (PC group) lower than control group. In both diabetic groups the salivary glucose level was not statistically different, but it was higher than salivary glucose level of the control group, so it is possible that this may have influenced the salivary flow rate. Increased salivary glucose concentrations in diabetics have also been reported by Karjalainen et al (1996). However, contrary to the present study, these authors observed that in diabetic patients with good metabolic control the salivary glucose level was similar to the control (Karjalainen et al, 1996). We observed a negative correlation between stimulated salivary flow rate and blood glucose concentrations, indicating that hyperglycaemia is a factor that influences the salivary flow rate. It has been reported that no relationship was found between a diminished salivary flow rate and the duration of diabetes, but that there is a relationship between the flow rate and blood glucose level (Stefaniotis and Donta, 1990).

In the present study, the normalisation of the glycaemia in the WC patients did not improve the salivary flow rate, once no difference with the PC was observed. According to Zachariasen (1992), diabetes affects the functional and structural integrity of the salivary glands, thus the glycaemic control is not efficient to normalise the flow rate. Dodds et al (2000) found that although salivary flow rates were lower in diabetic patients than in the control, they were not significantly different.

In the present study, the samples were obtained from persons living in three cities located in the interior

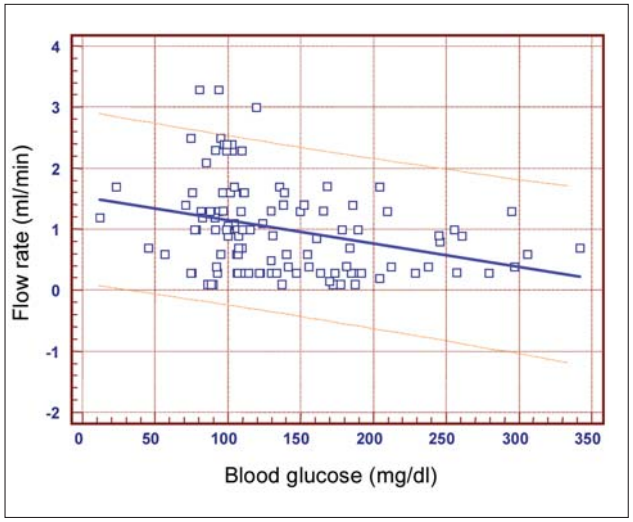

Fig 1 Dispersion diagram showing a negative correlation between the variable stimulated flow rate and the blood glucose concentration (r = -0.57; p = 0.0005).

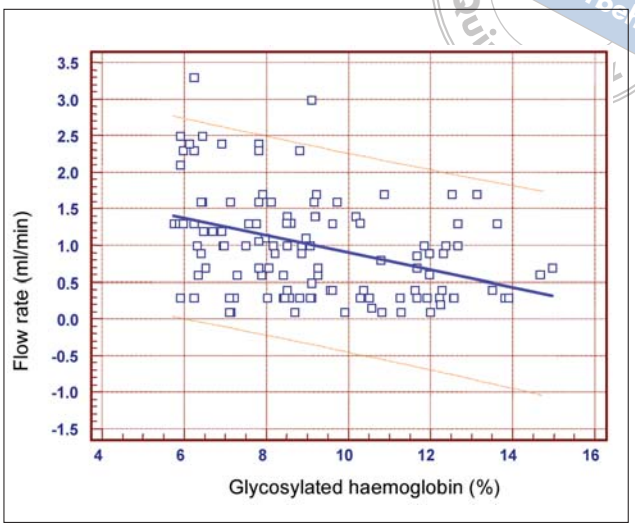

Fig 2 Dispersion diagram showing a negative correlation between the variable stimulated flow rate and the glycosylated haemoglobin (r = -0.61; p = 0.045).

of Santa Catarina state, aged from 31 to 80 years old, so they may have different nutritional habits. Saliva collected from these subjects presented a wide variation in pH, which may be seen by the slightly high standard deviation. It is possible that the mean pH level below 7.0 in stimulated saliva may be due to alteration in ion-content with age, as observed in the study by Chauncey et al (1987). The buffering capacity is defined as the ability of a solution to resist a change in pH when an acid or base is added. In the saliva of insulin-dependent diabetes, the buffer capacity has been reported to be higher than in the control (Kjellman, 1970), but this has not been confirmed by later studies (Tenovuo et al, 1986; Karjalainen et al, 1997). Several methods to determine the salivary buffering capacity are available, including colorimetric and electrometric methods. In the present study, we opted to mix saliva with an acid and measure the alteration of pH by a pH-meter. Some reports relate the reduction of the buffering capacity with the reduction of the flow rate (Collin et al, 1998; Lughetti et al, 1999). In this study no difference was observed in both initial pH and buffering capacity of saliva from diabetic patients compared with the control group.

The importance of verifying the xerostomia feeling has been emphasised (Dodds et al, 2000; Vernillo, 2001), as this feeling is considered one of the main buccal alterations in diabetics (Vernillo, 2001). In the present study, approximately 50% of the diabetic patients (WC or PC) related the feeling of dry mouth, a result that is in agreement with the report by Moore et al (2002).

The great variability of results reported in the literature may be explained by several factors, such as different study design, differences in sample populations, and different methods, nutritional habits, and geographical locations.

In conclusion, the present study shows that the diabetic patients from these three cities of the southern region of Brazil presented differences in salivary flow rates and salivary glucose level compared with the control, regardless of whether their diabetes was well- or poorly metabolically controlled.

ACKNOWLEDGEMENTS

This research was carried out in University of Oeste de Santa Catarina, Campus Joaçaba, SC, Brazil.

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