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Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India

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Abstract: Diabetes is known to influence salivary composition and function, eventually affecting the oral cavity. We thus evaluated saliva samples for levels of glucose, amylase and total protein, and assessed salivary flow rate in diabetics and healthy non-diabetics. We also analyzed these parameters with regard to duration and type of diabetes mellitus and gender, and aimed to assess the interrelationships among the variables included in the study. A total of 120 age- and sex-matched participants were divided into 3 groups of 40 each; the uncontrolled diabetic group, the controlled diabetic group and the healthy non-diabetic group. Salivary investigations were performed using unstimulated whole saliva. Mean salivary glucose levels were found to be significantly elevated in both uncontrolled and controlled diabetics, as compared to healthy non-diabetics. There were significant decreases in mean salivary amylase levels in controlled diabetics when compared to healthy non-diabetics. Other than salivary glucose, no other parameters were found to be markedly affected in diabetes mellitus. Further research is needed to explore the clinical implications of these study results. (J Oral Sci 52, 359-368, 2010)

Keywords: healthy non-diabetics; salivary parameters; uncontrolled and controlled diabetics; type of diabetes.

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Introduction

Diabetes mellitus is a complex multisystem disorder characterized by relative or absolute insufficiency of insulin secretion and/or concomitant resistance to the metabolic action of insulin on target tissues (1). The abundant oral manifestations seen in diabetes mellitus make it likely for dentists to come in contact with a significant number of diabetic patients. It is estimated that around the turn of the century, there were 40 million diabetics in India (2), and worldwide, the prevalence of diabetes is thought to have doubled between 1994 and 2010, with about 240 million people now suffering from the disease (3).

Oral fluid or whole saliva is a complex chemical milieu of teeth and oral soft tissues, consisting mainly of water, essential electrolytes, glycoproteins, antimicrobial enzymes and numerous other important constituents like glucose and amylase (3,4). Diabetes mellitus has been consistently documented to be associated with altered salivary composition and function. This disrupts the homeostasis of the oral cavity, making it susceptible to various oral ailments. Oral physicians hold the responsibility of recognizing significant associations between certain oral anomalies and diabetes mellitus (5).

Knowledge of the effects of diabetes on salivary composition and function remains equivocal. Basement membrane permeability of the parotid gland is reported to be higher in diabetes mellitus, and this results in raised percolation of components such as glucose, amylase and protein from blood, thus raising their levels in saliva (4,6-8). Many previous studies have found raised salivary glucose level in diabetes (2,5,9-18).

Salivary total protein is a vital component of saliva, with salivary proteins, predominantly comprising proline-rich proteins, mucin, amylase, immunoglobulins, statherin

and antibacterial factors, and these are responsible for most of the functions of saliva. However, reports regarding salivary amylase levels and salivary total protein levels in diabetics are controversial, as they have shown higher levels (18-20), lower levels (21,22), or comparable levels between diabetics and healthy non-diabetics (7,23-25).

With regard to salivary flow rates, most studies have shown either decreased levels in diabetics (11,18,22,25-29) or comparable levels among diabetics and healthy non-diabetics (14,19,20,23,30,31).

The oral manifestations seen in diabetes include gingivitis, gingival abscess, spontaneous gingival bleeding, periodontitis, erythema, paresthesia, burning and numbness of the oral mucous membrane, burning tongue, median rhomboid glossitis, geographic and fissured tongue, dental caries, plaque build-up, oral candidiasis and increased incidence of infections, altered wound healing, taste impairment, halitosis, dry mouth, sialosis particularly affecting the parotid gland, rare conditions such as lichen planus and lichenoid reactions, and enamel hypoplasia in children born of diabetic mothers, while there is a strong association between diabetic neuropathy and tooth loss and temporomandibular joint dysfunction in elderly diabetic patients (1,3,4-11).

Very few studies have been performed on salivary composition and function in diabetes, particularly in India; thus, the data to date are limited. Furthermore, the study results that have been reported are often contradictory in several aspects, and this suggests the need for further investigative studies. The relative inconsistency in the outcomes of various studies may be attributed to variations in the duration of diabetes, the age range of patients, and the metabolic control of diabetes.

The potential of saliva to aid in the monitoring of diabetes mellitus was therefore examined in the present study. Of all salivary parameters, glucose, total proteins, amylase and salivary flow rate appear to be the most closely related to the oral environment in diabetic patients. The ongoing controversy regarding the levels of these parameters in diabetics in the literature also spurred our interest.

The aims of this study were as follows: first, to estimate the constituents of saliva (glucose, amylase, total protein and salivary flow rate) in order to aid in reaching firm conclusions about their alterations in diabetics as compared to healthy non-diabetics; second, to compare and correlate these parameters in uncontrolled diabetics and controlled diabetics with regard to duration and type of diabetes mellitus, as well as gender; finally, to assess any significant correlations that may exist among the various parameters under consideration in the present study.

Materials and Methods

With approval from the institutional ethics committee, the present study was carried out at the Department of Oral Medicine and Radiology of S. P. D. College and Hospital, Sawangi-Meghe (Wardha), in collaboration with the Central Research Laboratory and Biochemistry Department of J. N. M. College, Sawangi-Meghe (Wardha). Study subjects were recruited from among outpatients of the Department of General Medicine of A. V. B. R. Hospital, Sawangi-Meghe (Wardha).

A total of 120 age- and sex-matched study subjects in an age range from 13 to 69 years were divided into 3 groups of 40 each: the uncontrolled diabetic group (Group 1; 22 males, 18 females; mean age, 48.50 ± 7.86 years; range, 26-62 years), the controlled diabetic group (Group 2; 25 males, 15 females; mean age, 49.50 ± 10.88 years; range, 13-69 years), and the healthy non-diabetic group (Group 3; 16 males, 24 females; mean age 46.12 ± 10. 25 years; range, 17-65 years) (Table 1).

The study groups were similar to those reported by Chavez et al. (32). Study groups were further divided according to disease duration: short duration diabetics

Table 1 Distribution of study participants by group, gender, age, and duration & type of diabetes mellitus

Variables		**Group 1** (40) Uncontrolled Diabetic group	**Group 2** (40) Controlled Diabetic group	**Group 3** (40) Healthy non-diabetic group
According to gender (Chi-square -test, *P* value = 0.12 - NS)	Male (*n*)	22	25	16
	Female (*n*)	18	15	24
According to Age (ANOVA test, *P* value = 0.32 - NS)	Mean ± SD (Age range in years)	48.50 ± 7.86 (26-62)	49.50 ± 10.88 (13-69)	46.12 ± 10.25 (17-65)
Duration of diabetes mellitus	Long duration diabetics	06	04	-
	Short duration diabetics	34	36	-
Type of diabetes mellitus	IDDM	11	8	-
	NIDDM	29	32	-

P value, probability value; SD, standard deviation; NS, not statistically significant

included those patients who have been diabetic for ≤ 6 years and long duration diabetics included those patients who have been diabetic for more than 6 years (32,33) (Table 1). Study groups were also subdivided according to the treatment they were taking for diabetes: insulin dependent diabetes mellitus patients (IDDM) included those patients who were on insulin and non-insulin dependent diabetes mellitus patients (NIDDM) included those patients who were on oral medications and diet control (Table 1).

In diabetics, fasting and post-meal blood glucose levels were evaluated and in healthy subjects, random blood glucose tests were performed in order to confirm them as non-diabetic.

Inclusion and exclusion criteria

1. There were 80 well-established cases of diabetes mellitus diagnosed by the medical faculty with features of polyuria, polydypsia and polyphagia and elevated blood glucose levels, as per the criteria established by the Expert Committee on Diagnosis and Classification of Diabetes Mellitus in 1998 (1). These cases were divided into patients with uncontrolled diabetes mellitus and those with controlled diabetes mellitus, as per blood glucose levels, to form Groups 1 and 2, respectively.
2. The third group was included as a control group, and comprised healthy non-diabetic subjects (Group 3) with no features of diabetes mellitus and blood glucose levels within normal limits.
3. Patients with severe diabetic complications, with any other systemic illnesses or on medications other than those for diabetes were excluded.

Blood glucose levels were taken as an indicator of metabolic control (34-37); the criteria were as shown in Table 2.

Initially, the study protocol was explained and informed consent was obtained from all participants. A thorough case history was then taken, followed by oral and general examination. Blood samples and unstimulated and stimulated salivary samples were collected for each participant.

Salivary sample collection and salivary flow rate estimation

Participants were instructed not to brush their teeth, or eat, drink or smoke 2 h before the time of saliva collection. If a participant was a denture wearer, the dentures were removed prior to saliva collection. Subjects were asked to spit out or swallow saliva that was already present in the mouth and samples collected in the initial 30 s were discarded. Salivary sample collection was performed in the morning between 8.00-11.00 a.m. with study subjects sitting upright in a comfortable position in calm and cool isolated room. Samples were collected in an ice-chilled graduated saliva collector by the spitting method for at least 5 min. Thus, unstimulated whole saliva was collected (2,11,15,21,23,26-28,32).

In order to measure stimulated salivary flow rate, 2% acetic acid was used as a stimulator for salivary secretion, and the was applied to the dorsum of the tongue with a cotton tip applicator at 30-s intervals to collect stimulated whole saliva for at least 5 min.

For both unstimulated and stimulated saliva, the volume of saliva collected was noted in milliliters and the time for which saliva collected was noted in min. Unstimulated salivary flow rates (UFR) and stimulated salivary flow rates (SFR) were then measured in terms of milliliters per minute (ml/min) (Table 3).

Unstimulated whole saliva samples were used for salivary estimations. One milliliter of each unstimulated saliva sample was centrifuged at 3,000 rpm for 20 min and clear supernatants were processed immediately for estimation of glucose, amylase and total protein (12,20,26,27,30,31) using a semiautomatic analyzer (Biotron BTR 830).

Materials used

1. Kit for glucose estimation (Glucose oxidase, End point assay), Autospan-Cogent, Span Diagnostics, Surat, India
2. Alpha-Amylase Kit (Direct Substrate Method, Kinetic Enzymatic), Crest Biosystem, Goa, India
3. Microprotein Estimation (Pyrogallol Red Dye, End point method), Accucare, Sarigam, India

Salivary glucose estimation

Salivary glucose estimation was performed using the glucose oxidase end-point method (2,11,18). Briefly, 1,000 μl of reagent solution was pipetted into each of 3 test tubes labeled 'Blank', 'Standard' and 'Test'. Then, 10 μl

Table 2 Criteria of blood glucose level as an indicator of metabolic control to categorize the patients as uncontrolled diabetics and controlled diabetics

Relationship with food (in mg/dl)	For controlled diabetes mellitus	For uncontrolled diabetes mellitus
Fasting blood glucose level	<140	≥140
2-Hour postprandial blood glucose level	<200	≥200

Table 3 Distribution of mean levels of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy non-diabetic group

Parameter	Group	Mean ± SD
Salivary glucose (mg/ml)	Uncontrolled group	8.09 ± 6.45
	Controlled group	7.64 ± 6.44
	Healthy group	1.89 ± 1.44
salivary amylase (u/ml)	Uncontrolled group	108.48 ± 6.37
	Controlled group	100.83 ± 60.77
	Healthy group	146.72 ± 10.70
salivary total protein (mg/dl)	Uncontrolled group	90.01 ± 44.22
	Controlled group	88.91 ± 49.71
	Healthy group	98.25 ± 49.59
USR (ml/min)	Uncontrolled group	0.18 ± 0.12
	Controlled group	0.18 ± 0.14
	Healthy group	0.21 ± 0.20
SFR (ml/min)	Uncontrolled group	0.51 ± 0.27
	Controlled group	0.48 ± 0.29
	Healthy group	0.57 ± 0.35

mg/dl, milligram per deciliter; u/ml, units per milliliter; ml/min, milliliters per minute; USR, unstimulated salivary flow rate; SFR, stimulated salivary flow rate

Table 4 Intergroup comparisons of mean levels of salivary parameters in the uncontrolled diabetic group, controlled diabetic group and healthy non-diabetic group

Parameter	Intergroup comparisons	Dunnett D test *P* value	Intergroup comparisons	*Z*-test *P* value
salivary glucose (mg/dl)	Uncontrolled group	0.00	Uncontrolled group	0.75
	with healthy group	S	with controlled group	NS
	Controlled group	0.00		
	with healthy group	S		
salivary amylase (mg/dl)	Uncontrolled group	0.10	Uncontrolled group	0.65
	with healthy group	NS	with controlled group	NS
	Controlled group	0.04		
	with healthy group	S		
salivary total protein (mg/dl)	Uncontrolled group	0.66	Uncontrolled group	0.91
	with healthy group	NS	with controlled group	NS
	Controlled group	0.59		
	with healthy group	NS		
USR (ml/min)	Uncontrolled group	0.54	Uncontrolled group	0.95
	with healthy group	NS	With Controlled group	NS
	Controlled group	0.57		
	with healthy group	NS		
SFR (ml/min)	Uncontrolled group	0.63	Uncontrolled group	0.56
	with healthy group	NS	with controlled group	NS
	Controlled group	0.31		
	with healthy group	NS		

S, statistically significant; NS, not statistically significant

of standard was added to the test tube marked as 'Standard', followed by 10 μl of test sample to the 'Test' test tube. These were mixed well and all the test tubes were kept in an incubator at 37°C for 10 min before aspiration. Reagent blank was first aspirated in the analyzer, followed by standard solution, for which the reading was noted, and finally, the test sample was aspirated and the reading was noted. Results were calculated and values were expressed as milligrams per deciliter (mg/dl) (Table 3).

Salivary amylase estimation

Salivary α-amylase estimation was performed using the direct substrate kinetic enzymatic method (18,38,39), the mean absorbance change per minute (Δ A/min) was calculated in terms of units per liter (u/l) (Table 3).

Salivary total protein estimation

Salivary total protein estimation was performed using pyrogallol red dye (40,41) by the end-point method, and results were calculated and values were expressed in terms of milligram per deciliter (mg/dl) (Table 3).

Table 5 Correlations between salivary parameters in the uncontrolled diabetic group

Pearson correlation test Parameter	Fasting blood glucose (mg/dl)	Post meal blood glucose (mg/dl)	Salivary glucose (mg/dl)	Salivary amylase (u/ml)	Salivary total protein (mg/dl)	USR (ml/min)	SFR (ml/min)
Salivary glucose (mg/dl)	*0.04 S	0.94 NS	-	*0.05 S	0.22 NS	0.55 NS	0.21 NS
Salivary amylase (u/ml)	0.22 NS	0.85 NS	*0.05 S	-	*0.00 S	0.14 NS	0.15 NS
Salivary total protein (mg/dl)	0.29 NS	0.48 NS	0.22 NS	*0.00 S	-	*0.03 S	*0.04 S
USR (ml/min)	0.46 NS	0.88 NS	0.55 NS	0.14 NS	*0.03 S	-	*0.00 S
SFR (ml/min)	0.41 NS	0.84 NS	0.21 NS	0.15 NS	*0.04 S	*0.00 S	-

Table 6 Correlations between salivary parameters in the controlled diabetic group

Pearson correlation test Parameter	Fasting blood glucose (mg/dl)	Post meal blood glucose (mg/dl)	Salivary glucose (mg/dl)	Salivary amylase (u/ml)	Salivary total protein (mg/dl)	USR (ml/min)	SFR (ml/min)
Salivary glucose (mg/dl)	0.11 NS	0.41 NS	-	0.82 NS	0.84 NS	0.11 NS	0.72 NS
Salivary amylase	0.90 NS	0.26 NS	0.82 NS	-	*0.01 S	0.58 NS	0.77 NS
Salivary total protein (mg/dl)	*0.01 S	0.31 NS	0.84 NS	*0.01 S	-	*0.02 S	*0.04 S
USR (ml/min)	0.51 NS	0.38 NS	0.11 NS	0.58 NS	*0.02 S	-	*0.00 S
SFR (ml/min)	0.65 NS	0.65 NS	0.72 NS	0.77 NS	*0.04 S	*0.00 S	-

Table 7 Comparison of salivary parameters in the uncontrolled diabetic group by gender

Parameters	**Gender**	***n***	**Mean ± SD**	**Student's *t*-test *P* value**
Salivary glucose (mg/ml)	Male	22	7.30 ± 5.84	0.39
	Female	18	9.06 ± 7.17	NS
Salivary amylase (u/ml)	Male	22	106.32 ± 67.61	0.86
	Female	18	111.11 ± 110.94	NS
Salivary total protein (mg/dl)	Male	22	77.51 ± 40.26	0.04
	Female	18	105.28 ± 45.11	S
USR (ml/min)	Male	22	0.20 ± 0.13	0.24
	Female	18	0.15 ± 0.10	NS
SFR (ml/min)	Male	22	0.59 ± 0.29	0.04
	Female	18	0.42 ± 0.21	S

n, no. of subjects; S, statistically significant; NS, not statistically significant

Statistical analysis

All study variables were statistically analyzed using the SPSS 10.00 software package. All values are expressed as means ± standard deviation (SD) and *P* (probability) values of ≤ 0.05 were considered to be significant; *P* values of ≤ 0.01 were considered to be highly significant. Diabetic groups were compared with the healthy non-diabetic group using Dunnett's d-test (Tables 4), and differences among the uncontrolled and controlled diabetic groups were analyzed by Z-test (Tables 4). Intragroup correlations were also carried out for each group by applying Pearson's correlation coefficient test (Tables 5 and 6).

For each study group, intragroup comparisons were performed according to duration, type and gender (Table 7) using Student's *t*-test.

Results

Considering the prevalence of diabetes mellitus and its oral manifestations, it has become of paramount importance to study the levels of some crucial parameters in diabetic saliva.

Salivary glucose

Mean salivary glucose levels were higher in the uncontrolled and controlled diabetic groups than in the healthy non-diabetic group and the differences were highly significant (Tables 3 and 4). Uncontrolled diabetics had higher mean salivary glucose levels than controlled diabetics (Tables 3 and 4).

Salivary amylase, total protein and flow rates

The findings were not significantly different when diabetics and healthy non-diabetics were compared for salivary amylase, salivary total protein and salivary flow rate (Tables 3 and 4), although mean salivary amylase levels were significantly lower in the controlled diabetic group than in the healthy non-diabetic group (Tables 3 and 4).

Intragroup correlations as assessed by Pearson's correlation tests

In addition to estimation of salivary parameters and intergroup comparisons, intragroup correlations were assessed for each study group by applying Pearson correlation coefficient tests; each salivary parameter in turn was examined for correlations with every other parameter analyzed. The results were as follows:

a. Significant positive correlations were observed in salivary glucose levels and salivary amylase levels in the uncontrolled diabetic group (Table 5).
b. Significant positive correlations were observed in salivary total protein levels with fasting blood glucose levels in the controlled diabetic group (Table 6).
c. Highly significant positive correlations were observed between salivary amylase levels and salivary total protein levels in both the uncontrolled and controlled diabetic groups (Tables 5 and 6).
d. Significant negative correlations were observed between salivary total protein levels and unstimulated and stimulated salivary flow rates in both the uncontrolled and controlled diabetic groups (Tables 5 and 6).

Duration and type of diabetes mellitus

No statistically significant intergroup or intragroup correlations were seen for the uncontrolled and controlled diabetic groups based on duration and type of diabetes mellitus.

Gender of participants

Each study group was analyzed for gender differences with regard to salivary parameters. Mean salivary total protein levels were significantly lower in uncontrolled diabetic males than in uncontrolled diabetic females (Table 7), while mean salivary stimulated flow rate was significantly higher in uncontrolled diabetic males than in uncontrolled diabetic females (Table 7).

Discussion

Oral pathophysiology and general diabetic complications are complex, and there is considerable heterogeneity within the diabetic population with regard to the development and progression of such complications.

Salivary glucose

Mean salivary glucose levels were clearly higher in diabetics when compared with healthy non-diabetics. For salivary glucose levels, the results of the present study are in accordance with most previous studies (2,5,9-18,42). However, Sharon et al. (23) previously showed elevated salivary glucose levels only in parotid saliva, while Marchetti et al. (43) reported no changes in salivary glucose levels in diabetics.

Normal glucose levels in saliva are 0.5-1.00 mg/100 ml, and do not significantly affect oral health or support the growth of microorganisms. However, higher salivary glucose levels favor the proliferation of microorganisms and enhance their colonization on teeth and oral mucous membranes. Glucose serves as a nutrient for candidal microorganisms and suppresses the killing capacity of neutrophils, which further accentuates colonization and likely consequences can be proposed as a result of these elevated salivary glucose levels in diabetes (1,4,15). Oral diseases that may be ascribed to the elevated salivary glucose levels include candidiasis, dental caries, gingivitis, periodontal disease, increased risk of infection, burning mouth, fungal infections, taste impairment and poor wound healing. Prolonged xerostomia may also be a contributing factor to these conditions.

The elevated salivary glucose level in diabetes also confirms the effects of diabetic membranopathy, which leads to raised percolation of glucose from blood to saliva (4,6,8), thus altering the salivary composition in diabetes mellitus.

Salivary amylase and salivary total protein

The significantly lower mean salivary amylase levels in controlled diabetics when compared with healthy non-diabetics seen in the present study supports the study by Yavuzilmaz et al. (21), who linked them to hormonal and

metabolic changes occurring in diabetic patients. However, the results were insignificant for uncontrolled diabetics in our study. In contrast, significant increases in salivary amylase levels have also been demonstrated in diabetics (2,18,19).

The importance of amylase in the adhesion of micro-organisms remains uncertain, although several studies have supported amylase as a potential factor in streptococcal adhesion to teeth and in plaque formation (44).

The present study revealed a positive correlation between salivary amylase levels and salivary glucose levels, in agreement with previous studies (2,19). Pal et al. concluded that higher glucose concentrations favored proliferation of microbes and amylase caused accumulation of plaque with consequent rises in total bacterial count in diabetics (2).

With regard to salivary total protein, the present study results are consistent with most previous studies (7,16, 17,19,20,23-25), with no significant differences evident between diabetics and non-diabetics. However, recent studies have reported higher salivary total protein levels in diabetics (2,18,21), while Streckfus et al. (22) estimated significant lower protein concentrations in diabetics and emphasized protein utilization by other biochemical metabolic pathways an overall systemic response to glucose intolerance. Insulin is known to have the potential to alter protein metabolism (19).

A significantly positive correlation was observed between salivary total protein levels and fasting blood glucose levels in the controlled diabetic group.

Another major observation was the highly significant positive correlation between salivary amylase levels and salivary total protein levels in both diabetic groups, as previously reported by Pal et al. (2). Salivary amylase is one of the major components of salivary total protein; hence, it can be speculated that variations in salivary amylase levels will simultaneously and proportionately affect salivary total protein levels. The present positive correlation thus appears to be justified.

Salivary flow rate

For salivary flow rates, no significant differences were seen between diabetics and healthy non-diabetics, which corroborates several previous studies (14,19,20,23,30,31). However, other studies demonstrated significant decreases in flow rates in diabetics (11,18,22,25-29). Nonetheless, diabetics tended to show lower salivary flow rates than healthy non-diabetics in our study.

Decreases in salivary flow rate or oral dryness occurring in diabetes can be multifactorial, 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, local inflammation and irritation in the oral cavity, infections, metabolic disturbances, and neuropathy affecting the salivary glands, and may be due to drug therapy for diabetes or concomitant drugs (,2,14,20,23,28,29,45). In addition, the present study found a significant negative correlation between salivary flow rates and salivary total protein levels; this observation is the same as that reported by Lopez et al. (18) in diabetic children. In contrast, Reutering et al. (24) showed a significant positive correlation between salivary flow rates and salivary total protein levels in resting saliva.

Duration of diabetes mellitus

Surprisingly, there were no significant findings with regard to duration of diabetes mellitus. No correlations were previously found between salivary glucose levels, salivary flow rate and disease duration (11,15,27), although other studies inferred that with increased duration of diabetes, glucose values in saliva decreased as a result of fatty infiltration in the acini and diabetic microangiopathy of salivary glands (2,26). This was seen in the controlled diabetic group in the present study, while reverse was seen in the uncontrolled group. However, in both the uncontrolled and controlled diabetic groups, shorter disease duration tended to be associated with higher salivary levels of glucose, amylase and total protein and stimulated flow rates when compared with longer disease duration.

Type of diabetes mellitus

No significant differences were seen between the IDDM and NIDDM diabetic groups, which is in agreement with other studies (15,16,21,22,25).

The unique observation made here is that, in general, IDDM subjects tended towards higher levels in all the salivary estimations than NIDDM subjects, except for a few exceptions in the controlled diabetic group.

Gender of participants

Significantly lower mean salivary total protein levels and higher mean salivary flow rates were seen in diabetic males than in diabetic females in the uncontrolled diabetic group, which was similar to the results of other studies (11,18) and contradicted the results reported by Chavez et al. (27).

For salivary glucose levels, no significant differences were seen between gender, and this was in agreement with Darwez et al. (15), although higher salivary glucose levels have been reported in males when compared with females (13,15,20).

For salivary glucose, salivary amylase and salivary total

protein, diabetic males tended to show lower levels than diabetic females, and the reverse was found for salivary flow rates. Conversely, in the healthy group, there was a striking predilection towards higher levels in males than in females for all salivary parameters.

The present study included critical salivary parameters, namely glucose, amylase, total protein and salivary flow rate, in order to study the effects of diabetes on salivary composition and function. The study also gave due consideration to the level of metabolic control, duration and type of diabetes mellitus, and gender. In addition, the study focused on most possible interrelationships amongst these variables. It could be an important consideration to study these interrelationships as they may guide us to explore the mechanisms underlying various oral pathologies manifesting in diabetic patients.

Although many studies have been carried out on salivary parameters in diabetes, our study is one of the very few that have assessed all the salient parameters and variables affecting them in a single cohort. Hence, despite the abundance of literature, the study outcomes to date are not suitable for comparison.

It is generally accepted that diabetes influences salivary composition and function. Our study found that no salivary parameters other than salivary glucose were affected in diabetes. Thus, we were unable to confirm any discernible differences in salivary composition and function among diabetics and non-diabetics. Presumably, with such a fine margin of distinction, the clinical relevance is questionable.

Moreover, no significant differences were seen between uncontrolled and controlled diabetics, but they were diversely affected in some aspects. In this context, the differences between the subgroups require additional comparative study, particularly since the literature is very limited with regard to duration and type of diabetes, as very few researches have taken these factors into consideration. We compared salivary parameters based on duration and type presuming that they would provide some fundamental information in terms of the oral manifestations seen in diabetes.

Furthermore, it is unclear why so much diversity was seen in the levels of salivary parameters with regard to gender.

The present study has several potential limitations, such as the small sample size. We were unable to recruit a large number of long duration diabetics and insulin dependant diabetics in our study span. Although the results are not entirely conclusive quantitatively, the study made some novel observations that will unquestionably contribute to providing a platform for further research.

A particularly important issue is the vivid correlations elucidated in this study, and as we cannot propose plausible explanations for them, there is space for further research.

The observations derived from this study require more comprehensive evaluation with emphasis on broader representation. We believe that these new perspectives will provide insight in reaching a clear consensus regarding changes in salivary composition and function in diabetes mellitus.

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