

The influence of salivary components on dental caries in patients with type I diabetes mellitus

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Abstract

Saliva is considered as a intricate oral fluid that keeps the oral cavity hydrated and immune from foreign components. The incidence of dental caries depends on the quantity of saliva secretion. The presence of glucose in excess and reduced salivary concentration has been noted in Type I diabetes Mellitus (T1DM) patients, thereby indicating an increased prospect of dental caries associated with T1DM patients.

Aims and Objectives: The study is intended to assess the connection between prevalence of dental caries and the components of saliva like, salivary flow rate, buffer capacity, pH, streptococcus mutans count, salivary glucose level, salivary α -amylase in T1DM children with age groups between 08-16years. The comparison was done with age and gender to controls involved in the study.

Materials and Methods: A case controlled study was designed incorporating 8-16 year old group of children with T1DM and controls were group same age and sex. Salivary flow rate, pH, DMFS Scores, buffering capacity, salivary glucose level, salivary α -amylase and streptococcus mutans count were analysed for all the subject groups. Salivary factors were compared by using Unpaired 't' test, whereas chi square test was used to analyse salivary buffering capacity. The association between salivary components and caries prevention between T1DM subjects and non T1DM subjects was done by Pearson correlation test.

Results: The study results showed DMFS scores in permanent dentition, indicated for extraction and filled teeth surfaces affected by caries in primary dentition (DMFS) score in T1DM were 2.610 and non T1DM was 2.6 respectively. The mean of salivary flow rate in T1DM was 0.261ml/min and non T1DM was 0.661ml/min, thus it was lesser in T1DM patients. The T1DM salivary pH showed an average of 6.51 which was lower in comparison with 7.31 in non T1DM patients. The mean salivary glucose level was found to be 2.310mg/dl in T1DM and 0.721 in non T1DM patients, therefore indication a higher salivary glucose levels in T1DM patients. The mean salivary α -amylase levels were found to be lower in T1DM patients, as it showed 120U/ml in T1DM and 148U/ml in non T1DM patients. The mean of streptococcus mutans count showed no difference between the groups, thereby T1DM patients showing 2.71X10⁵ CFU/ml and non T1DM showing 2.91X10⁵ CFU/ml respectively.

Conclusion: The study found a statistical significance between salivary flow rate, pH, buffering capacity and α -amylase levels lower in T1DM patients but salivary glucose levels were higher than compared to non T1DM patients. The T1DM patients salivary buffering capacity had a significant association with dental caries. Increased attribution to dental caries associated with salivary components was noted in T1DM cases than non T1DM control group.

Keywords: Saliva, Diabetes, Children.

Introduction

Saliva plays a major role in hydrating the oral cavity and maintaining the integrity of oral mucosa. Unlike other body fluids it is challenging to study saliva due to the intra and inter individual variations in composition and physical properties.¹ various salivary substances have direct and indirect role in the onset of caries.²⁻⁴ T1DM is characterised by hyperglycemia due to insufficient secretion of insulin caused by autoimmune illness and genetic factors.⁵ In India, the incidence of T1DM is 4.2/100,000 population per year.⁶ The oral manifestations of DM include xerostomia, taste impairment, non-inflammatory enlargement of salivary glands, tooth decay, periodontal changes to fungal infections, oral lichen planus and fissured tongue.⁷ Reduced salivary flow has been reported in diabetics however many have failed to confirm this relationship.⁸⁻¹¹ Scientific evidence predicts more acidic saliva associated in Type 1 diabetics.¹²⁻¹⁴ The acidic salivary pH in diabetic children may be attributed either to microbial activity or to a decrease of bicarbonate.¹⁵ The depletion in the buffer capacity among diabetic patients may be due to decrease in the salivary production causing low salivary output which

triggers acidic salivary secretion leading to amplified severity of dental caries.¹⁶

α -Amylase, a calcium-containing metalloenzyme is found in acquired enamel pellicle and dental plaque, it helps to promote adhesion of *S. mutans* to the tooth surface. Bacterial adhesion to α -Amylase on the tooth may be considered undesirable if it contributes to dental plaque formation.¹⁷ increased glucose levels and low salivary output in T1DM patients have been hypothesized to explain the possibility of having a higher incidence of oral problems.¹⁸ scientific reports on oral health and salivary status in Type 1 diabetic patients are frequent in the dental literature but inconclusive.

Thus the present study is aimed to assess the relation between caries prevalence (defs and DMFS) and the salivary components like salivary output, pH, buffering ability, salivary glucose and amylase level and streptococcus mutans count in Type 1 Diabetics with 8-16 year old children. The study was framed to establish the level of glycemic control of 8-16 year old children with glycosylated hemoglobin (HbA1c) level, to determine dental caries using defs and DMFS index, to estimate the

constituents of salivary output, pH, buffering ability, salivary glucose and amylase level and streptococcus mutans count in diabetics as compared to healthy non-diabetics and to assess the relationship of the caries experience and the salivary components with the level of metabolic control in Type 1 Diabetes Mellitus patients.

Materials and Methods

This is an *in vivo* comparative cross sectional study. The data was collected from 96 children aged between 8-16 years with T1DM at Bangalore Diabetes Hospital. Data from healthy subjects were collected from the patients who visited the Department of Pedodontics and Preventive Dentistry, M.S. Ramaiah Dental College and Hospital, Bengaluru. Group I involved children with T1DM and group 2 involved healthy children.

Saliva and Dental Caries Associated Components Were Evaluated as below

1. Assessment of dental caries using DMFS /defs: The examination of dental caries was carried out using mouth mirror, CPI probe under natural light/ torch, as per the guidelines of American Dental Association for Type 3 examination. A trained assistant recorded DMFS/defs based on the criteria developed by WHO 1997.44
2. Salivary secretion: After 1 hour of consumption of any food or liquid, unstimulated saliva (5ml) was received between 9am to 12pm. The time at which saliva was collected were noted in minutes and millilitre value was given for salivary volume. Millilitres per minute (ml/min) measurement was taken to measure unstimulated salivary flow rate (UFR).
3. Salivary pH: Lutron pH-206, a digital pH meter was used to compute salivary pH.
4. Salivary buffering capacity: Buffering capacity of the collected saliva was determined using Dentobuff® Strips.
5. Estimation of salivary glucose: The analysis was done by using Beckman Coulter Glucose, REF - OSR 6521 reagent was used. To estimate salivary glucose, 1 ml of saliva was used and the sample was directly placed in the container and loaded in the equipment to estimate glucose values
6. Estimation of salivary α -Amylase: The analysis was done using Beckman Coulter α -Amylase, REF - OSR 6006 reagent is a standard test kit that was used for the estimation of salivary amylase.
7. *Streptococcus mutans* count: *Streptococcus mutans* count was assessed after culturing in Mitis Salivarius Bacitracin (MSB) agar.

Microbiological Procedure

Preparation of MSB Agar Plates

1. 18 grams of *Mitis Salivarius agar*, 30 grams of sucrose and 3 grams of *Agar Agar* were measured and mixed into 200 ml of distilled water in a conical flask
2. The flask was sealed and autoclaved

3. 400 μ l of bacitracin and 200 μ l of potassium tellurite were added to the mix before cooling
4. The mix was poured into sterile petri plates in the laminar air flow chamber and allowed to set
5. The plates were then stored in the refrigerator at 4°C

Sample Processing and Culture

1. Each salivary sample was diluted in phosphate buffered saline and homogenized in a vortex mixer. The amount of dilution of the sample with the phosphate buffer was decided by comparing the turbidity that arises to that of the Mc-Farland Nephelometer standards. This was done to ensure uniform dispersion of the organism in the buffer and to help prevent any discrepancy that might have occurred from collecting differing quantities of the sample.
2. The sample was then inoculated onto a Mitis Salivarius Bacitracin (MSB) Agar by pipetting 200 μ l of the buffer containing sample onto the petri plates which was then spread onto the plate uniformly with the help of the sterile inoculating glass rod
3. The MSB agar plates were incubated in an anaerobic jar for 48 hours at 37°C in an incubator.
4. *S. mutans* colonies were identified by colony characteristics appearing as dark navy blue, convex or raised, shiny colonies.
5. *S. mutans* counts were recorded by a colleague (to ensure blinding) in colony forming units using a digital colony counter unit.

Statistical Analysis

Data was collected and compiled into an Excel spread sheet and records were computed using SPSS version 20.0 software.

1. For continuous variables the results were given by descriptive data as mean, standard deviation and standard error of mean
2. Unpaired 't' test : this test was chosen to find significant difference concerning T1DM group and non T1DM group for all the salivary variables except for buffering capacity.
3. Chi- square test was chosen for the categorical variables like buffering capacity.
4. Intergroup comparison was done by using Pearson correlation to find the correlation between the salivary components and the caries experience involving the T1DM and non- T1DM group.

Results

The study results demonstrated that the mean DMFS/defs scores of group 1 and group 2 was similar. The mean *S. mutans* count was found similar in both the groups. Statistical significance was observed from unpaired 't' test showing lower values in cases with salivary flow rate, pH and α -amylase than control groups. Cases showed increased salivary glucose values than control groups leading to statistical significant when assessed using unpaired 't' test. A p-value < 0.05 was considered to be statistically significant.

Table 1: Comparison of salivary parameters, DMFS/ defs scores between cases and controls

	T1DM (N=96)	Non T1DM (N=96)	p value
	Mean $\pm$ SD	Mean $\pm$ SD	
DMFS/defs	2.61 $\pm$ 0.92	2.59 $\pm$ 0.91	0.875
Flow rate (ml/min)	0.37 $\pm$ 0.10	0.67 $\pm$ 0.13	0.001
pH	6.43 $\pm$ 0.34	7.36 $\pm$ 0.38	0.001
Salivary glucose(mg/dl)	2.37 $\pm$ 0.23	0.72 $\pm$ 0.24	0.001
Salivary amylase (U/ml)	119.04 $\pm$ 7.20	146.91 $\pm$ 4.40	0.001
S.mutans (X10 ⁵ CFU/ml)	2.79 $\pm$ 0.53	2.93 $\pm$ 0.53	0.054

Table 2: Associating buffering capacity between the cases and controls

(N=96)	Buffering capacity	p value
Diabetic	Intermediate (N=58) High (N=38)	
Non-diabetic	Intermediate (N=3) High (N=93)	

Value for buffering capacity

Low: < 4

Intermediate: 4.5-6.5

High: > 6.5

A lower buffering capacity was found among the diabetic group. A statistic significance ($p < 0.05$) was obtained when compared to the non T1DM group.

Table 3: Correlation of DMFS/defs with the salivary parameters among cases and controls

Pearson correlation		Diabetic group DMFS/defs	Non diabetic group DMFS/defs
Flow rate (ml/min)	Pearson Correlation	-0.05	-0.17
pH	Pearson Correlation	-0.14	-0.11
Buffer capacity	Pearson Correlation	-0.22*	-
Salivary glucose (mg/dl)	Pearson Correlation	0.10	-0.166
Salivary amylase (U/ml)	Pearson Correlation	0.02	0.29**
S.mutans (x10 ⁵ CFU/ml)	Pearson Correlation	0.14	0.046

* indicates significant

** indicates highly significant

Buffering capacity was negatively correlated (-0.228 times) with the DMFS/defs scores in the T1DM group and a statistically significant ($p < 0.05$) was noted. Salivary amylase was positively correlated (0.292 times) with the DMFS/defs scores in non T1DM group and a statistically significant ($p < 0.05$) was obtained.

Discussion

Juvenile Diabetes or adult onset diabetes is associated with pH changes and buffering capacity of saliva in adolescents.¹⁹⁻²¹ In the present study a significant reduction in the pH of patients with diabetes was observed as compared to non-diabetic patients, a significant reduction in buffering capacity was also observed.

In diabetic children, the acid pH may be related to microbial activity or reduced bicarbonate with flow rate.¹² Nevertheless, diabetes may cause changes in salivary glands thus contributing to rise in pathogenic bacteria.²² A study conducted by Nadia Al-Rawi and Sulafa El-Samarrai

demonstrated diabetic patients with lower pH and the statistical significance was obtained between the control group and diabetic patients. The changes in buffer capacity is evident in T1DM patients as it will lead to the production of more acidic saliva causing incidence of dental caries with increased severity in adults.¹⁶

It has been hypothesized that hyperglycemia in T1DM patients provides explanation for higher incidence of dental caries.²¹ Current study with T1DM patients, showed glucose concentration were considerably higher as compared to the control group. The study also showed that the mean salivary glucose levels to be higher in diabetics (mean = 2.3mg/dl) when compared with control group (mean = 0.723mg/dl).

The salivary glands acts as filters of blood glucose and are altered by hormonal and neuronal regulation.²³ The persistent hyperglycemia leads to microvascular changes in the salivary gland blood vessels. This results in increased leakage of glucose from salivary ductal cells thus increasing the salivary glucose concentration.²⁴⁻²⁶

Salivary components are altered by metabolic, nutritional and neurological abnormalities, medications like diuretics, antihistaminics, antihypertensives etc.²⁷ The present study findings showed that T1DM with the HbA1c levels ranging between 8.5-10% with poor metabolic control, demonstrated a similar overall DMFS scores in the diabetic individuals (mean = 2.61) when compared to the non-diabetics (mean = 2.59). The results also showed considerable reduction of unstimulated salivary flow rates in diabetic individuals compared to the non-diabetics with statistically significant ($p < 0.05$), and our values were in agreement with the other studies.^{12,28-30}

Reduced salivary flow rate or oral dryness in patients with diabetes can be multifactorial, either due to fatty infiltration into the salivary glands or physical alteration of mucosal cells subsequent to dehydration due to polyuria or microvascular disease, local inflammation and infections in the oral cavity, metabolic disturbances and neuropathy affecting the salivary glands, and may be due to diabetic medications or concomitant medications.^{11,31}

Conclusion

The present case control study demonstrated that both T1DM and non T1DM groups showed no difference in caries severity but the salivary flow rate, pH, and amylase was lower and salivary glucose was higher in T1DM cases compared to the non T1DM controls. Dental caries was associated with salivary buffering capacity as it was low in T1DM cases than controls. The salivary amylase was significantly linked with dental caries in non T1DM control group. Dental caries in T1DM group was triggered by discrepancy related to various components of saliva.

General dental practitioners should be aware of the oral manifestations concerned with T1DM in children. Strict preventive measures like topical fluoride application, fit and fissure sealants and oral hygiene educational programmes can be instituted. T1DM patients should be screened by the Pediatric dentist at the younger age itself so that individualized treatment plan can be formulated, based on the oral health status.

Conflict of Interest: None.

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