Evaluation of Salivary Components and Dental Plaque in Relation to Dental Caries Status in Type 1 Diabetes Mellitus

Manjushree R[1], Latha Anandakrishna[2], Keerthi Prasad KS[3], Ashmitha K Shetty[4]

Abstract

Purpose: To evaluate the dental caries prevalence in Type 1 Diabetes Mellitus (T1DM) as compared to healthy children and, to assess the salivary components (flow rate, glucose, α-Amylase, *Streptococcus mutans*) and dental plaque in relation to their dental caries status.

Methods: Dental caries were estimated by using the DMFS index and dental plaque by Sillness and Loe plaque index. The following methods were used to assess the salivary components; draining method to determine the flow rate; glucose oxidase peroxidase method for glucose; substrate method for α-Amylase, Mitis Salivarius Bacitracin agar was used to culture *Streptococcus mutans*.

Results: Caries prevalence was significantly lower in T1DM. In the diabetic group, a significant positive correlation was found between DMFS value and plaque, DMFS value and salivary glucose, and also with DMFS value and salivary α-Amylase. A significant negative correlation was found between the DMFS value and the unstimulated salivary flow rate. The multivariate regression analysis demonstrated that decrease in the unstimulated salivary flow rate to be significantly associated with increasing DMFS values.

Conclusion: The caries prevalence was found to be low in T1DM when compared to the healthy children, the cause for it being related to the low plaque scores. Low caries prevalence could also be due to the restriction of sucrose in their diet.

Clinical significance: As clinicians, along with restoring the smiles of a child patient, we have to retrospectively analyze the factors involved in the causation of dental caries. Educating the parents and the child in regard to this will help prevent the occurrence of any new carious lesion.

Keywords: Dental caries, Dental plaque, Saliva, Type 1 Diabetes Mellitus.

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Introduction

Type 1 Diabetes Mellitus (T1DM) is a metabolic disorder characterized by a hyperglycemic state resulting from a definitive deficiency in insulin secretion.[1] The incidence rate in India is about 3.4/100,000 population per year.[2]

Studies in the literature show controversy with the caries prevalence in (T1DM). High caries prevalence in T1DM was found in a study done by Ferizi,[3] however study done by Gupta[4] showed low caries prevalence. Ferizi[3] reported that the changes in the salivary components are responsible for the high caries prevalence in T1DM, whereas Gupta[4] mentioned that the influence of diabetic diet on plaque and acidogenic bacterial microflora to be responsible for the low caries prevalence.

Salivary components that have been listed in the causation of dental caries in T1DM include salivary flow rate, salivary glucose, Salivary α-Amylase, and *Streptococcus mutans*. Salivary α-Amylase, an enzyme that hydrolysis α 1, 4 linkages of starch to glucose and maltose, is also been found in dental plaque. This enzyme has been related to dental caries as it can lead to the adherence of *S. mutans* to the tooth surface.[5] *S. mutans* are the most cariogenic bacteria[6] that have the ability to create a low pH environment favorable for the progression of caries.[7] Thus the action of Salivary α-Amylase is related to both, the production of glucose and the adhesion of *S. mutans* to the tooth surface.

Regarding salivary glucose levels, Ferizi[3] has stated that the activity of microorganisms is affected by an increased salivary glucose levels, thereby increasing the caries activity. On the contrary, Bolgu[8] has stated that T1DM patients have a low prevalence of dental caries, because of the omission of sucrose in their diet. As sucrose breaks down rapidly into its constituent parts,

[1]Department of Pedododntics and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bengaluru, Karnataka, India

[2,4]Department of Pedodontics and Preventive Dentistry, Faculty of Dental Sciences, MS Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India

[3]Department of Preventive and Community Dentistry, Bangalore Institute of Dental Sciences, Bengaluru, Karnataka, India

Corresponding Author: R Manjushree, Department of Pedododntics and Preventive Dentistry, Bangalore Institute of Dental Sciences, Bengaluru, Karnataka, India, Phone: +91 8971481039, e-mail: manjushreerajappa@gmail.com

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that is 50% glucose and 50% fructose, less glucose production can be expected in diabetics following sucrose restricted diet, and is therefore been related to low caries prevalence.

Reports on the prevalence of dental caries in T1DM and its association with the salivary components have been found in the dental literature but most of them are inconclusive. Due to the contradictory ideas, the present study aimed at evaluating the dental caries status in T1DM as compared to the healthy children, and to assess the salivary components (flow rate, glucose, α-Amylase, *S. mutans*) and dental plaque in relation to their dental caries status.

The null hypothesis was that there is no difference in the dental caries prevalence in T1DM as compared to the healthy children in relation to their salivary components and dental plaque.

METHODS

Study Design and Setting

This cross-sectional study was conducted on 80 patients of age group 12–16 years. Group 1 consisted of 40 patients from the Bangalore Diabetes Hospital, who were diagnosed as having T1DM and were following sucrose restricted diet for ≥3 years. The metabolic control of diabetic patients was determined by the level of glycosylated hemoglobin, who all had HbA1c levels ≤7% (good control). The diabetics were following the sucrose-restricted diet that had been advised by the same hospital. Also, the hospital had been educating these patients to follow oral hygiene practices for maintaining good oral hygiene. Oral hygiene practices followed by group 1 patients included brushing teeth twice daily, once in the morning and night, and gargling just after food to rinse off any food debris present.

Group 2 consisted of 40 outpatients of the same age having ≥1 carious lesion who visited the Department of Pedodontics and Preventive Dentistry, M S Ramaiah Dental College, Bengaluru.

Children in the group 2 did not have any pre-existing systemic diseases and were not on any medications.

Prior to the case history and oral examination, the parental informed consent of each participant was obtained based on their willingness to participate in the study. As the T1DM patients were on a sucrose-restricted diet, the daily sugar intake was obtained by using a 24-hour recall diet frequency chart. The participants in both the groups were categorized into excellent, good, and watch out zone based on the obtained sugar scores. Checkmarks were placed in the frequency column of each item as long as they were eaten 20 minutes apart. (Score- 5 for a liquid form of sugar, score- 10 for a solid and sticky form of sugar, score- 15 for a slowly dissolving form of sugar). A sweet score of 5 or less was interpreted as "excellent"; 10 as "good" and 15 or more as "watch out" zone.[9]

A power analysis was established by G*power, version 3.0.1 (Franz Faul University, Kiel, Germany). A sample size of 77 subjects which is rounded off to 80 (40 in each group) would yield 80% power to detect significant differences, with an effect size of 0.309 and a significance level of 0.05.

Dental Caries and Plaque Assessment

The clinical examinations for all the participants were done by a single principal investigator R to avoid interexaminer variability, and a recording assistant was present throughout the examination to aid in filling the case sheet. All participants were asked to brush their teeth with a toothbrush and toothpaste prior to the dental examination. The dental examination of both groups was carried out in the dental set up, without disclosing the participant's group to avoid bias in recording the DMFS scores between the groups. The participants were examined by using a dental mirror, dental explorer, and World Health Organization (WHO) periodontal probe. DMFS scores were derived from the standard indices recommended by the WHO Oral Health Survey Basic Methods manual for dentition charting of caries (2013).[10]

Dental plaque scores were obtained by using Silness and Loe plaque index on mesial, buccal, distal, and palatal/lingual surface of teeth: 16, 12, 24, 32, 36, and 44. The nominal scale for evaluation of plaque score was: 0 for excellent hygiene; 0.1–0.9 for good hygiene; 1.0–1.9 for fair hygiene; 2.0–3.0 for poor hygiene.[11]

Assessment of Salivary Components

Resting saliva was collected with the free-flowing method for 5 minutes.[12] Unstimulated saliva was collected during the morning hours at least half an hour after brushing and, the subjects were asked to refrain from eating or drinking until the collection of saliva. The subjects were asked to relax for a few minutes before the saliva collection. Saliva was obtained with the children seated on the chair with their heads being slightly bent forward. Saliva was allowed to drip down the lip to the sterile tubes. The collected volume of saliva was measured in milliliters per minute (mL/min). As the patient spat the saliva into a sterile container, the unstimulated salivary flow rate was determined. After collecting 5 mL of saliva from each participant, the tubes were placed on ice and immediately sent to the laboratory. The saliva was centrifuged at 2,500 rpm for 5 minutes to obtain salivary samples free of large particulate debris.[13]

The glucose oxidase peroxidase (GOD-POD) end-point method was used to estimate glucose levels in the saliva. Into the three test tubes labeled "Blank," "Standard," and "Test" of each saliva sample, 1,000 µL of reagent solution was added. Later, 10 µL of the standard was added to the test tube labeled as "Standard," followed by 10 µL of a test sample was added to the "Test" test tube. After mixing vigorously, the test tubes were placed at 37⁰ C for 10 minutes in an incubator. Later, the reagent "Blank," standard solution, and the test were placed in the automatic analyzer and the readings were expressed as milligrams per deciliter (mg/dL).[14]

CNPG3 (2-Chloro4-Nitrophenyl-α-Maltotrioside) substrate method (kinetic enzyme assay) was used to estimate α-Amylase levels in the saliva. The ability of α-Amylase to catalyze the hydrolysis of starch to maltose was used as the principle to estimate α-Amylase. One part of saliva was diluted in 99 parts of saline and, the diluted 25 µL of saliva sample was taken in a test tube for the estimation. About 1.0 mL of reagent solution was added into each of the three test tubes labeled as "Blank," "Standard," and "Test" of saliva, and all the three test tubes were placed in an automatic analyzer. The readings were noted and expressed in terms of units per liter (U/l).[14]

To Mitis Salivarius (MS) agar, 0.2 U/mL of bacitracin and 10% sucrose were added to obtain a high selection of *S. mutans*. About 0.5 mL of salivary samples were diluted in a solution of sterile phosphate buffered saline with 0.05 M and pH 7.3. On Mitis Salivarius agar supplemented with bacitracin and sucrose, the diluted 15 µL of the salivary sample was plated. The plates were kept in a 5% carbon dioxide (CO_2) incubator at 37⁰ C for 2 days. Later, the *S. mutans* that appeared as raised, convex, undulate, opaque, pale blue colonies with a granular "frosted glass" appearance were differentiated from the other spices. A colony counter was used to count the *S. mutans* count. The obtained *S. mutans* colony forming units per milliliter was recorded as CFU/mL.[15] Berkowitz criteria were used to score the number of colonies obtained. Score 0 for no growth, Score 1 for 1–10,[3] Score 2 for 10^3 -10,[5] Score 3 for ≥ 10^5 CFU per mL of saliva.[16]

Statistical Analysis

Data was entered in the excel spreadsheet; descriptive statistics such as mean; standard deviation were calculated. Statistical testing between groups (diabetics and their nondiabetics) was carried out using the paired *t*-test for continuous data. Pearson's correlation coefficient (r) was used to correlate DMFS with plaque scores and the clinical parameters. Multivariate analysis was used to find the association of independent variables with DMFS using SPSS (Statistical Package for Social Sciences) version 20.

[(IBM SPASS statistics) IBM Corp. released 2011]. The 95% confidence intervals (CI) were calculated; a p-value of 0.05 was considered to be statistically significant.

RESULTS

T1DM had statistically significantly lower caries experience when compared to healthy children. The mean DMFS score was 1.75 in group 1 and 3.93 in group 2. T1DM had a statistically significant lower dental plaque score when compared to the healthy children. The mean dental plaque score was 0.38 in T1DM and 1.39 in the healthy group.

The T1DM children had statistically significant lower unstimulated salivary flow rates, higher salivary glucose, and salivary α-Amylase when compared to the healthy children. The mean salivary flow rate in group 1 and group 2 was 0.19 and 0.39 mL/min, respectively. The mean salivary glucose levels in group 1 and group 2 was 10.15 mg/dL and 2.20 mg/dL, respectively. The mean salivary α-Amylase in group 1 and group 2 was 144.07 U/l and 103.42 U/l, respectively. However, there were no differences in S. mutans counts between the groups (Table 1).

In T1DM, significant positive correlations (r) was found between DMFS value and plaque score (r = 0.84), DMFS value and salivary glucose (r = 0.905) and also with DMFS value and salivary α-Amylase (r = 0.88). Significant negative correlation was found between DMFS value and the unstimulated salivary flow rate (r = -0.92). In group 2, significant positive correlations (r) was found between DMFS value and plaque score (r = 0.92), DMFS value and salivary glucose (r= 0.89) and also with DMFS value and salivary α-Amylase (r = 0.90). Significant negative correlation was found between DMFS value and the unstimulated salivary flow rate (r = -0.90) (Table 2).

The multivariate regression analysis demonstrated that a decrease in the unstimulated salivary flow rate was significantly associated with increasing DMFS values in T1DM, whereas in nondiabetics increase in the plaque scores were significantly associated with increasing DMFS values (Table 3).

DISCUSSION

In our study T1DM patients demonstrated lower DMFS scores when compared to the nondiabetics which is consistent with the results obtained by Gupta et al.[4]

There was a significant reduction of unstimulated salivary flow rates in the T1DM group which is consistent with the study done by Ben-Aryeh.[17] Decrease in salivary flow rate causing oral dryness in diabetics can be multifactorial; it can either be due to fatty infiltration of cells into the salivary glands or due to physical alteration of mucosal cells. Local inflammation, irritation, infections, metabolic disturbances, neuropathy affecting the salivary glands, and drug therapy are the other factors that lead to dehydration in them.[18] Malicka et al.[19] have stated that, following the hyperglycemia and glycosuria, body fluids are lost causing dehydration in the body which subsequently reduces the secretion of saliva.

We found the mean salivary glucose levels to be significantly higher in the T1DM group that is consistent with the study done by Belazi.[20] The persistent hyperglycemia causes microvascular changes in the blood vessels leading to the alterations in the basement membrane permeability of salivary glands. This results in increased leakage of glucose from the ductal cells of the salivary gland into the whole saliva.[20] Miller[21] has stated that the increased glucose content in the whole saliva is a simple reflection of the blood glucose levels, derived from ultrafiltrate of plasma by three mechanisms, that is, passive diffusion, active transport, and ultrafiltration.

Salivary α-Amylase was found to be significantly higher in the T1DM group which is consistent with the study done by Lopez.[13] Piras[22] has mentioned that the long-term insulin treatment may account for the increased expression of α-Amylase in human diabetic parotid glands. The action of salivary α-Amylase is related to both, the production of glucose and the adhesion of S. mutans to the tooth surface. As the vast majority of carbohydrates that T1DM patients consumed was made up of starch, the action of salivary α-Amylase on starch has to be considered. Relating the action of salivary α-Amylase to the reduction in the flow rate in diabetics, a reduction in the activity of salivary α-Amylase could be expected. Fenoll et al.[23] has stated that a decrease in the salivary flow rate declines the pH of the saliva. As the optimum pH of 7 is required for the activity of salivary α-Amylase, the enzyme activity is expected to be low with the decreased pH present in the diabetics.[24] Thus, salivary α-Amylase could not have had any crucial role in the causation of dental caries.

Dental plaque was found to be significantly lower and, had a positive correlation with dental caries in the T1DM group. As the major risk factor for the development of caries is bacterial plaque and, as the diabetic group had starch as the vast majority of carbohydrates, the action of plaque bacteria on the type of diet has to be considered. Regarding this, Stephan and Hemmens[25] found that starch, in comparison with mono and disaccharides, was the poorest substrate for acid formation by pure cultures of plaque bacteria, which explains the reason for the low prevalence of dental caries in the T1DM group as compared to the healthy children.

As a part of case history, the dietary habits between the groups were recorded. The sweet score was excellent in the T1DM group as all 40 subjects were on a sucrose-restricted diet. Among the healthy children, 18 subjects were categorized as "good" and 22 subjects under "watch out zone," indicating that they all consumed a diet with high sucrose content. As Sucrose is the sole substrate in the synthesis of extracellular glucans, a major component of the structural inter-microbial matrix of dental plaque,[26] dietary type has to be given importance while considering the plaque score and S. mutans count. The mean S. mutans count in diabetics was similar to the mean S. mutans count in the non-diabetic group. Zero[27] has stated that S. mutans plaque extracted from sucrose-containing cultures had markedly enhanced demineralization potential compared with glucose-grown plaque. Dibdin[28] has stated that sucrose-mediated synthesis of glucans is associated with caries-associated virulence, wherein it increases the porosity of plaque, permitting deeper penetration of dietary sugar into the biofilm, and greater acid production immediately adjacent to the tooth surface.[26] Thus, the pioneer studies by Dibin and Guggenheim state that the sucrose-containing diet plays an important role in the causation of dental caries. In the present study, we found low caries prevalence in the diabetic children following sucrose-restricted diet.

This study states the importance of dental plaque in both, children with T1DM and healthy children. Despite salivary components being altered in the T1DM group; the caries prevalence was found to be lower than that of the healthy children which can be attributed to their good oral hygiene practices. Also, the most important factor to be considered is their sucrose-restricted diet; the restriction of this known arch-criminal in the diet could be a factor that can be associated with the low caries status in the T1DM group.

Table 1: Comparison of the groups using independent student's t-test

		Mean	S.D	Mean diff	p-value
DMFS	Diabetic	1.75	0.71	-2.17	0.00*
	Non-diabetic	3.93	3.12		
Plaque score	Diabetic	0.38	0.16	-1.01	0.00*
	Non-diabetic	1.39	0.75		
Flow rate (mL/min)	Diabetic	0.19	0.04	-0.19	0.00*
	Non-diabetic	0.39	0.07		
Salivary glucose (mg/dL)	Diabetic	10.15	2.86	7.95	0.00*
	Non- diabetic	2.20	0.92		
Salivary α-amylase (U/l)	Diabetic	144.1	6.63	40.6	0.00*
	Non-diabetic	103.4	4.31		
Strep. mutans count (CFU/mL)*10^5	Diabetic	4.1	0.49	0.11	0.37
	Non-diabetic	4.21	0.61		

*$p < 0.05$ was considered to be statistically significant;
D, standard deviation; Mean diff, mean difference

Table 2: Pearson's correlation of DMFS index with clinical parameters

		Diabetic		Non-diabetic	
		r value	p-value	r value	p-value
DMFS	Plaque score	0.84	0.00*	0.92	0.00*
	Flow rate	-0.92	0.00*	-0.90	0.00*
	Salivary glucose	0.90	0.00*	0.89	0.00*
	Salivary α-amylase	0.88	0.00*	0.90	0.00*
	Strep. mutans count	0.09	0.55	0.14	0.37

*$p < 0.05$ was considered to be statistically significant

Table 3: Multivariate regression analysis

	Unstandardized coefficients		Standardized coefficients	p-value
	B	Std. error	Beta	
Diabetic				
(Constant)	6.89	5.04		0.18
Plaque score	-0.32	0.75	-0.077	0.67
Flow rate	-14.05	5.49	-0.876	0.01*
Salivary glucose	0.082	0.06	0.333	0.21
Salivary amylase	-0.022	0.03	-0.203	0.44
Strep. mutans count	0.005	0.08	0.004	0.95
Non-diabetic				
(Constant)	-15.79	22.84		0.494
Plaque score	3.638	1.70	0.882	0.04*
Flow rate	-9.982	11.81	-0.239	0.40
Salivary glucose	-1.591	1.27	-0.472	0.22
Salivary α-amylase	0.196	0.25	0.271	0.45
Strep. mutans count	0.425	0.34	0.084	0.22

*$p < 0.05$ was considered to be statistically significant

Conclusion

- The caries prevalence in T1DM was found to be lower than that of the healthy individuals, the reason for it being correlated with low plaque scores. Low plaque scores in diabetic patients can be attributed to their better oral hygiene practices when compared to healthy children.
- Low caries status in T1DM could also be due to the sucrose-restricted diet. Since caries is a multifactorial disease, dietary type should also be considered while analyzing the caries status.

- As clinicians, along with restoring the smiles of a child patient, we have to retrospectively analyze the factors that have played role in the causation of dental caries irrespective of healthy children or children with special health care needs. Educating the parents and the child in this regards will help prevent the occurrence of new lesion in the future.

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