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Oral Microbial and Salivary Alterations Associated with Type 1 Diabetes Mellitus in Children: A Case-Control Study from Erbil City

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ABSTRACT

Background and objective: Type 1 diabetes mellitus (T1DM) affects the pH, flow rate, and content of saliva, which increases the risk of dental caries. In comparison to their matched healthy controls, this study aimed to assess the association between dental caries, salivary traits, and Streptococcus mutans levels in children with T1DM.

Materials and Methods: A case-control study was conducted with 20 children with T1DM and 20 age- and sex-matched healthy controls. Clinical dental examinations assessed the Ageless DMFT (A‑DMFT) index, and saliva samples were analyzed for secretion rate, pH, and S. mutans using quantitative real-time PCR.

Results: There were no significant differences in A‑DMFT scores or salivary secretion rates between the groups. However, the T1DM group had significantly lower salivary pH (7.00 vs. 7.20, $p = 0.002$) and higher S. mutans levels (2.73×10^5 vs. 3.25×10^4 CFU/mL, $p = 0.012$). A strong positive correlation between A‑DMFT and S. mutans was found ($\rho = 0.724$, $p < 0.001$). No significant correlation was observed between A‑DMFT and saliva secretion rate ($\rho = 0.183$, $p = 0.257$). In logistic regression analysis, lower salivary pH ($p = 0.005$) and higher S. mutans levels ($p = 0.023$) were independently associated with T1DM status.

Conclusion: Children with T1DM had higher levels of S. mutans and lower salivary pH than controls, indicating an increased risk for dental caries. The results imply that the elevated cariogenic potential shown in this group may be caused by variables other than salivary secretion rate.

Keywords: Dental caries; Diabetic children; real-time PCR; Salivary status; Streptococcus mutans

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders and a major global health issue characterized by a decrease in insulin production or impaired insulin action, resulting in hyperglycemia.[1] High blood sugar results in the metabolic alterations and cause complications and organ failure over time. The prevalence of diabetes mellitus increase all-aged groups and is estimated to reach 4.4% by 2030.[2] Most cases of DM are Type 2 Diabetes Mellitus (T2DM) but the most health complications appear in children with Type 1 Diabetes Mellitus (T1DM) which is an autoimmune condition.[2] In T1DM, the autoimmune process can also affect other organs that cause additional complications.[3]

Saliva from the salivary glands helps maintain oral health by balancing demineralization and remineralization, thereby preventing cavities. The mechanism by which diabetic patients experience a higher risk for dental caries is related to hyperglycemia which is linked to higher salivary glucose levels, reduced secretion, and changes in buffering capacity, affecting pH, remineralization, and flow.[4] Also, frequent carbohydrate intake increases their risk for dental caries. On the other hand, Children with T1DM often experience reduced saliva flow, lower buffering capacity, and higher levels of harmful bacteria like Streptococcus mutans and Lactobacillus.[5] Poor diabetes control worsens these factors, increasing the risk of dental caries. Thus, diabetes significantly impacts the oral cavity, especially in children with T1DM.[6]

Saliva plays a key role in protecting against dental caries by maintaining oral health through its flow, composition, and various components. It aids in microbial clearance, forms oral pellicles for microbial binding, enhances microbial killing, and provides nutrients. Salivary proteins, such as proline-rich proteins, mucins, and cystatins, protect tooth surfaces, promote remineralization, and prevent demineralization.[7] Saliva buffering action helps neutralize acids, maintaining pH balance and reducing caries risk.[8] Streptococcus mutans, a key bacterium in dental caries, is commonly found on occlusal surfaces and has been linked to higher caries risk. Many studies associate high S. mutans levels with increased caries.[9]

Since T1DM produces several physiological changes, it is crucial to understand salivary and caries status in order to prevent dental caries, and Erbil residents are no exception. It appears that no studies have been conducted in Erbil, in the Kurdistan area of Iraq, to evaluate cariogenic bacteria in children with T1DM. In order to make progress in terms of oral health promotion, and raise awareness and improve behaviors among children with T1DM, it has been decided to conduct a study to explore the influence of T1DM on dental caries and salivary status.

METHODS

Study Design and Setting

This case-control study was carried out in Erbil City at the Galiawa Learning Center for Diabetic Care. Utilizing the Power and Sample Size Calculation software (Vanderbilt University Department of Biostatistics, Nashville, TN, USA) and current literature, the sample size was established. A statistical power of 90% was targeted, while 0.05 was set as the significance threshold (alpha). Consequently, each group's anticipated sample size was 20, for a total sample size of 40 children.

This study included 20 children (10 males, 10 females) aged 6-10 years with T1DM diagnosed for at least one year, and 20 age- and sex-matched healthy controls. Exclusion criteria included children receiving treatments affecting salivary function (e.g., antidepressants, chemo/radiotherapy), those using mouthwash or undergone oral prophylaxis in the past 1 month, or those with severe cognitive impairments or who were uncooperative. Written informed consent was obtained from the parents or legal guardians.

Tools for Data Collection and Study Procedure

Two tools were adopted by the researchers:

First tool: Oral clinical examination

Under natural light, participants sat in a movable chair and had a clinical dental examination. Dental caries experience was assessed using the Ageless DMFT (A-DMFT) index. This unified measure combines dmft and DMFT values weighted by the number of primary and permanent teeth and adjusted by the square root of the individual's age.[10] The assessment was conducted using various equipment, including cotton (Aslanli, Turkey), cloth or paper hand towels, a portable dental chair, disposable dental mirrors and explorers (Atlas, Iran), disposable gloves (Prime Quality, Malaysia), and disposable face masks (Han Chuan Soar, China).

Second tool: Saliva sampling

Before saliva collection, participants were in-

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structed to avoid eating or drinking (except water) for at least one hour. They were also asked to remain calm and seated in a relaxed position during the procedure. To stimulate salivation, participants chewed paraffin wax. The sequence of tests for each participant was as follows: 1) Salivary secretion rate measurement, 2) Salivary pH assessment, and 3) S. mutans testing in laboratory.

Assessment of Salivary Flow Rate

Saliva secretion was measured using paraffin chewing gum, as described by Singh et al.[11] The patient was instructed to refrain from eating for at least an hour and was seated in an upright, relaxed position. After chewing the paraffin gum for 30 seconds, the patient either expectorated or swallowed the accumulated saliva. They then continued to chew the gum for 5 minutes, with saliva collected into a measuring sterile cup. The chewing duration was adjusted according to the participant's secretion rate and shortened if the rate was high or extended if it was low. After 5 minutes, the amount of saliva was measured and reported. Salivary secretion rates are categorized as follows: 0 for normal (≥ 1.1 ml/min), 1 for low (0.9 – 1.1 ml/min), 2 for very low (0.5 – 0.8 ml/min), and 3 for extreme low or xerostomia (< 0.5 ml/min).

Estimation of salivary pH

The salivary pH was evaluated using the GC Saliva-Check BUFFER.[12] A pH test strip was immersed in the sample of stimulated saliva for 10 seconds and then removed. The color change on the strip was compared with the provided testing chart, as shown in Figure 1.

Figure 1. pH testing chart

Evaluation and Enumeration of S. mutans Using Quantitative Real-Time PCR

Bacterial genomic DNA was extracted from saliva samples using a commercial DNA extraction kit (iNtRON, Korea) according to the manufacturer's instructions. The extracted DNA was assessed for quality and concentration using a NanoDrop1000 Spectrophotometer (Lantech, Korea), with samples considered suitable for further analysis if the A260/A280 ratio was above 1.7 and concentrations exceeded 20 ng/µL. To quantify S. mutans levels, a standard curve was created using DNA from known concentrations of S. mutans isolated from patients, with six-fold dilutions prepared for colony-forming unit (CFU) equivalents. Specific primers targeting the glucosyltransferase B)gtfB(gene of S. mutans (Forward: 5′-CTACACTTTCGGGTGGCTTG-3′, Reverse: 5′-GAAGCTTTTCACCATTAGAAGCTG-3′) were used.[13] The quantitative real-time polymerase chain reaction (qPCR) contained 10 µL Synergy Brands (SYBR) Green Premix Ex Taq™ (Takara), 0.4 µL of each primer (10 µM), and 1 µL of extracted DNA, with the final volume adjusted to 20 µL using distilled water. Amplification was performed under the following conditions: initial denaturation at 94°C for 4 minutes, followed by 40 cycles of 94°C for 20 seconds, 55.4°C for 20 seconds, and 72°C for 33 seconds. The threshold cycle (Ct) values were used to quantify S. mutans DNA in the samples, which were then converted to bacterial CFU per mL of saliva based on the standard curve. The bacterial load was categorized as follows: 0 for less than 104 CFU/mL (Very low or zero amount of mutans streptococci in saliva, represented only about 5% of the tooth surface colonization),1 for 104-105 CFU/mL (Low levels of mutans streptococci in saliva, represented about 20% of the tooth surfaces colonization), 2 for 105-106 CFU/mL (High

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amount of mutans streptococci in saliva, represented about 60% of the tooth surfaces colonization), and 3 for greater than 106 CFU/mL (Very high amounts of mutans streptococci in the saliva, representing more than 80% of the tooth surfaces colonization).[14]

Statistical Analyses

Statistical analyses were performed using SPSS (version 27). Normality of continuous variables was assessed using the Shapiro–Wilk test and showed non-normal distribution; therefore, non-parametric tests were applied. Between-group comparisons were conducted using the Mann–Whitney U test and Fisher's exact test, correlations were evaluated with Spearman's rho, and multivariable logistic regression was performed to identify independent predictors of T1DM. Statistical significance was set at $p < 0.05$. No missing data were identified in the dataset, and all collected observations were included in the final statistical analysis.

RESULTS

Baseline characteristics of participants with T1DM and healthy controls, Table 1. Gender distribution was balanced in both cohorts: the T1DM group comprised 10 males and 10 females, while the control group included 12 males and 8 females, with no statistically meaningful disparity (p = 0.525). Median ages were closely aligned between groups, 9.00 (8.00–10.00) years for the T1DM group and 9.50 (7.50–10.00) years for controls, showing no significant variation (p = 0.989). Dental health, as measured by the DMFT index, yielded comparable results: the median A-DMFT score was 5.00 (3.50–6.50) in the T1DM group, slightly higher than the control group's 4.00 (3.00 –5.00), though this difference lacked statistical significance (p = 0.242). Similarly, salivary flow rates were nearly identical across groups, with median values of 0.85 (0.65–1.20) mL/min in T1DM participants and 0.95 (0.65–1.40) mL/min in controls (p = 0.470).

Table 1. Demographic and Clinical Characteristics of Study Population

Parameters		Type 1 Diabetes Mellitus (n=20) No. (%)	CONTROL (n=20) No. (%)	P value	Effect size
Gender	Male	10 (50)	12 (60)	0.525	0.101
	Female	10 (50)	8 (40)		
Age		9.00 (8.00–10.00)	9.50 (7.50–10.00)	0.989	0.002
A-DMFT		5.00 (3.50–6.50)	4.00 (3.00–5.00)	0.242	0.185
Secretion Rate		0.85 (0.65–1.20)	0.95 (0.65–1.40)	0.470	0.114
pH		7.00 (6.80–7.10)	7.20 (7.00–7.35)	0.002*	0.489
St. mutans (CFU/ml)		2.73×10^5 (2.84×10^4–1.10×10^6)	3.25×10^4 (1.24×10^4–9.12×10^4)	0.012*	0.398

* indicate statistically significant differences ($P < 0.05$)

Notably, saliva pH levels diverged significantly between groups. The T1DM group exhibited a lower median pH of 7.00 (6.80–7.10) compared to 7.20 (7.00–7.35) in controls, a difference that reached statistical significance with a large effect size (p = 0.002, Effect size = 0.489). Furthermore, S. mutans concentrations in saliva were significantly higher in T1DM patients, with a median of 2.73×10^5 CFU/mL (2.84×10^4–1.10×10^6), compared with 3.25×10^4 CFU/mL (1.24×10^4–9.12×10^4) in the control group (p = 0.012, Effect size = 0.398), indicating a moderate to large effect size. In the T1DM group, 15% had less than 104 CFU/mL of S. mutans, while in the control group, 20% of participants had less than 104 CFU/mL of S. mutans. A higher percentage of the T1DM group (25%) had more than 106 CFU/mL compared to none in the control group. The difference in the distribution of S. mutans levels between the two groups was statistically significant (P =

0.009, Effect size = 0.512), reflecting a large effect size, as shown in Table 2.

Table 2. Distribution of Streptococcus Mutans Classes by Group

St. mutans		**Case**	**Control**	**P value Fisher exact test**	**Effect size**
Categories	**CFU/ml**	**Frequency (%)**	**Frequency (%)**		
0	Less than 10^4 (5% of the tooth surface colonization)	3 (15)	4 (20)	0.009*	0.512
1	Between 10^4 and 10^5 (20% of the tooth surfaces colonization)	4 (20)	12 (60)		
2	10^5 and 10^6 (60% of the tooth surfaces colonization)	8 (40)	4 (20)		
3	More than 10^6 (More than 80% of the tooth surfaces colonization)	5 (25)	0 (0)		
Total		20 (100)	20 (100)		

* indicate statistically significant differences (P < 0.05)

In both groups, the majority of participants had a normal (35%) or low (31.6%) secretion rate, with no significant difference between the two groups (P = 1.00). However, 5.3% of the T1DM group had severely reduced salivary flow (xerostomia) compared to none in the control group, Table 3.

Table 3. Distribution of Saliva Secretion Rate Categories by Group

Saliva		**Case**	**Control**	**P value**	**Effect size**
Categories	**Secretion Rate / Minute**	**Frequency (%)**	**Frequency (%)**		
0	Normal	6 (31.6)	7 (35)	1.00	0.164
1	Reduced (0.9 – 1.1)	6 (31.6)	6 (30)		
2	Low (0.5 – 0.8)	7 (31.6)	7 (35)		
3	Xerostomia (< 0.5)	1 (5.3)	0 (0)		
Total		20 (100)	20 (100)		

Fisher exact test

A moderate positive correlation was observed between the A-DMFT score and S. mutans count in saliva (Spearman's ρ = 0.724, p < 0.001), indicating a significant relationship where higher DMFT scores were associated with elevated levels of S. mutans. This correlation was found in a sample of 40 participants from both case and control groups, as shown in Figure 2. Within the case group, a stronger positive correlation was noted (Spearman's ρ = 0.737, p < 0.001), further supporting the association between A-DMFT and S. mutans. Also, the control group (T1DM) exhibited a moderate positive correlation (Spearman's ρ = 0.654, p = 0.002), suggesting a more moderate link between DMFT and S. mutans in this population. Both correlations were statistically significant at the 0.01 level, highlighting a consistent and reliable relationship between dental caries and S. mutans presence in both groups. The analysis revealed a very weak positive correlation between DMFT and saliva secretion rate (Spearman's ρ = 0.183), which was not statistically significant (p =

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0.257). These findings suggest that there is no significant association between the A-DMFT index and saliva secretion rate in the study sample (40 participants). The correlation was not significant for control and case group as well, (p = 0.219) and (p = 0.525), respectively.

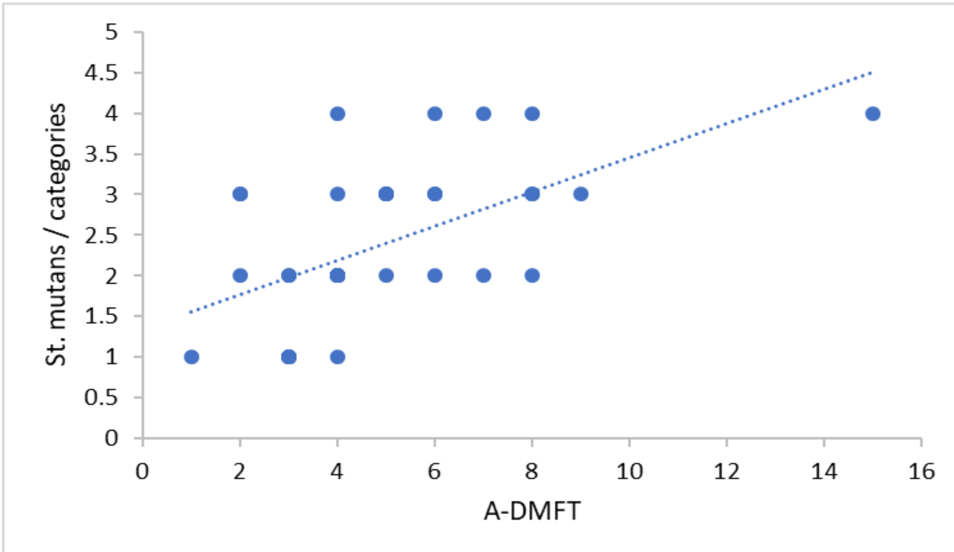

Figure 2. Correlation Between DMFT Score and Streptococcus mutans Count in Saliva across the Type 1 Diabetes Mellitus and Control Groups

A multivariable logistic regression analysis was performed to determine whether salivary and clinical variables were independently associated with T1DM status after adjustment for potential confounders. After adjustment, lower salivary pH was independently associated with T1DM (Adjusted OR = 0.0003, 95% CI: 0.000–0.086, p = 0.005). Additionally, higher levels of S. mutans were significantly associated with T1DM status (Adjusted OR = 0.085, 95% CI: 0.010–0.707, p = 0.023), as shown in Table 4. Other variables including age, gender, secretion rate, and A-DMFT were not statistically significant predictors in the adjusted model.

Table 4. Multivariable Logistic Regression Analysis for Factors Associated with T1DM

Variable	B	SE	Adjusted OR	95% CI for OR	p-value
Gender (male)	0.172	0.942	1.188	0.19 – 7.53	0.855
Age	-0.257	0.393	0.773	0.36 – 1.67	0.513
Secretion rate	1.215	1.199	3.370	0.32 – 35.36	0.311
Salivary pH	-8.258	2.960	0.001	0.001 – 0.086	0.005*
S. mutans (>10^5 CFU/ml)	-2.467	1.082	0.085	0.010 – 0.707	0.023*
A-DMFT	0.055	0.266	1.056	0.63 – 1.78	0.837

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A-DMFT	0.055	0.266	1.056	0.63 – 1.78	0.837

DISCUSSION

This study offers significant new data about the association between salivary parameters (including levels of Streptococcus mutans), dental caries, and T1DM in children in Erbil City. Despite no significant variations in DMFT scores or salivary secretion rates when compared to healthy controls, the results show significant changes in the oral microenvironment of children with T1DM, as seen by decreased salivary pH and increased S. mutans levels.

The absence of significant differences in A-DMFT scores between T1DM and control groups contrasts with some prior studies reporting higher caries prevalence in children and adolescents with T1DM, for instance, a meta-analysis study concluded that both DMFT and dmft were significantly higher in D1TM group than the non-T1DM group.[15] Patients between the ages of 10 and 15 had noticeably higher DMFT index values, according to another case-control experimental investigation, which likewise indicated that this was caused by inadequate metabolic management.[5] Additionally, A clinical examination study concluded that adolescents with T1DM had more filled teeth, fewer decayed teeth, and a higher mean DMFT score than the healthy controls.[16] Conversely, a comparison analysis revealed DMFT and dmft scores of 32 children with T1DM and 32 age and gender matched controls did not differ significantly.[17] Regional variables that were not evaluated in this study, such as dietary patterns, fluoride intake, or dental hygiene practices, could be the cause of this disparity.

Notably, the T1DM group exhibited significantly lower salivary pH and higher S. mutans levels, aligning with evidence that hyperglycemia promotes the growth of pathogenic bacteria and altering salivary composition.[4] Furthermore, a similar case control research found that children with diabetes who had poor metabolic control were far more likely to have Lactobacillus and S. mutans than children with good metabolic control.[5] As T1DM alters the composition of saliva and glycemic management, it is associated with an increased risk of dental caries. S. mutans, cariogenic Lactobacillus species, and the combination of Streptococcus species all contribute to the development of dental caries.[18] An experimental investigation on the salivary pH in forty children with T1DM indicated that the control group had a higher mean salivary pH, however this difference was not statistically significant.[19] The finding of our study in terms of pH, align with the finding of other studies.[20,21] Low pH can result from the composition of saliva being directly impacted by the degree of metabolic regulation.[22] Even though salivary flow rates were similar, the lower pH in children with T1DM probably indicates a decreased buffering capacity, which may accelerate enamel demineralization.[23]

A moderate positive association between A-DMFT and S. mutans highlights the bacterium's participation in the etiology of caries. The A-DMFT index and salivary secretion rate did not correlate, which runs counter to research that linking hyposalivation to dental caries.[24] Although the secretion rates in both groups in this study were within normal ranges (mean: 0.98–1.09 mL/min), this suggests that the caries risk in this cohort may be driven by qualitative changes in saliva (e.g., pH, glucose content) rather than flow rate. This aligns with findings in non-diabetic children where salivary pH, rather than salivary flow rate, showed a significant correlation with caries status.[25] This highlights the importance of comprehensive salivary profiling in T1DM management. However, a saliva sample only captures bacterial counts at a particular time, and it is well known that tooth decay develops over a long period, during which bacterial counts can change in response to changes in the oral environment.

This study has several limitations. HbA1c levels were not measured, which restricts interpretation of how metabolic control relates to salivary and microbial changes. Saliva was collected at a single time point, which may not represent long-term oral conditions. Only S. mutans was assessed, without evaluating other cariogenic bacteria such as Lactobacillus. In addition, key confounding factors including diet, fluoride exposure, and oral hygiene, were not controlled. The relatively small sample size and case-control design further limit the generalizability of the findings, so future longitudinal studies are needed.

CONCLUSION

According to this study, children with T1DM were at a higher risk for dental caries because their salivary pH was lower and their levels of S. mutans were considerably higher than those of healthy controls. Salivary secretion rates and A-DMFT scores, however, did not significantly differ across the groups. The results imply that the elevated car-

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iogenic potential shown in this group may be caused by variables other than salivary secretion rate, such as the changed salivary composition in children with type 1 diabetes.

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Conflict of Interest

The authors declare no conflicts of interest.

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