Association between Dental Caries, Cariogenic Bacteria and Salivary Characteristics among Type – II Diabetics

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Abstract

Background: Diabetes can significantly affect oral health, particularly regarding dental caries, salivary pH, and salivary microorganism composition. While many studies have examined the relationship between blood sugar and periodontal diseases, few have examined the effect of diabetes on dental caries. **Objective:** To examine salivary changes, the prevalence of dental caries, and its association with bacterial counts and socio-demographic factors among patients with type 2 diabetes mellitus. **Methods:**A total of 100 subjects, with 50 individuals diagnosed with Type 2 Diabetes and 50 without, were matched according to sex, from whom salivary samples were analysed for pH using pH strips and a culture of Streptococcus mutans and Lactobacillus acidophilus was done and estimated. Clinical examination included DMFT according to WHO 2013 criteria, and root caries was scored as present or absent. **Results:** The root caries was significantly higher among diabetics (0.2±0.5 vs 0.08±2.7). The salivary pH was higher among males (6.5) in comparison to females (6.3) (p=0.033). In contrast, no differences were found in the number of teeth affected by coronal caries, salivary pH, S. mutans, or L. acidophilus between the two groups. In the final regression models, age significantly predicted dental caries, while blood sugar significantly predicted root caries. **Conclusion:** Notably, dental caries in the study population was found to be influenced by age, while salivary pH was influenced by gender and root caries by blood sugar levels. It is crucial to raise awareness about the need for routine oral health examinations for people with high blood sugar levels because diabetics are more prone to dental cavities.

Keywords: Bacteria, Dental caries, Saliva, Type 2 diabetes.

Introduction

Globally, the incidence of non-communicable illnesses is rising, especially in developing countries.(1). This poses agrave public health concern that needs careful analysis and focused preventative action. In recent years, the relationship between oral health and systemic conditions has emerged as a pivotal area of research, shedding light on the complex relationships influencing overall well-being. Diabetes mellitus (DM) and dental caries are the two most common conditions.(1). The common factor between them appears to be sugar consumption emphasizing the importance of addressing dietary factors to reduce the burden of both diseases as described through the common risk factor approach.(2).

DM is characterized as a metabolic dysfunction that disrupts the proper processing of carbohydrates, fats, and proteins. This disturbance in metabolism is linked to elevated blood sugar levels, resulting from either impairment in the action of insulin hormones or insufficiency(3).Based on the pathologic process that leads to hyperglycaemia it is classified under two broad categories - Type 1 and Type 2. Type 1 DM (DM1) is an autoimmune disease caused by the destruction ofbeta cells of the pancreas and hence also called insulin-dependent diabetes,usually affecting children and young adults(4). Whereas, diabetes mellitusType 2 (DM2)presentsavicious cycle initiated by impaired insulin secretionoranincreasein insulin resistance and glucose production among older adults. Based on the predictions of the IDF Diabetes Atlas (2021), 10.5% of the adult population (20-79 years)are diabetics, and roughly half of those affected are unaware of their condition.By 2045, approximately 783 million adults, or 1 in 8, will be living with diabetes with a 46% projected increase. Type 2 diabetes, contributes to 90% of cases, it is mainly influenced by socio-demographic, genetic and environmental factors(5). In India, the prevalence of Type II diabetes is 8.9%, and this rate is rising rapidly, primarily attributed to the increasing prevalence of overweight/obesity and unhealthy lifestyles(6, 7). Patients with diabetes often experience hyperglycemia, a condition managed through the use of oral antidiabetic medications such as biguanides (metformin) and sulfonylureas (glimepiride and glibenclamide).Long-term use of these drugs might cause dry mouth and a reduction in salivary flow(8).Complications arising from uncontrolled diabetes mellitus (DM) can impact various body systems, with heart failure and coronary heart disease being the primary long-term concerns(9, 10).Additionally, individuals with DM face a notable prevalence of oral health issues, including salivary gland dysfunction, xerostomia, heightened vulnerability to bacterial, viral, and fungal infections, tooth loss, taste impairment, periapical abscesses, lichen planusandburning mouth syndrome(1). Moreover, the deficiency in insulin hormones among diabetics contributes to alterations in both the quantity and composition of saliva, resulting in hyposalivation and an increased concentration of glucose in the saliva(11). The reduction in salivary secretion among individuals with DM leads to a diminished salivary flow rate, impacting the acid-base balance, commonly known as the Potential of Hydrogen (pH), within the oral cavity(12). Saliva plays a crucial role in maintaining pH by eliminating carbohydrates metabolizable by bacteria and neutralizing acids produced by bacteria. Consequently, a low salivary flow rate can result in a decreased salivary pH, leading to acidity. An acidic salivary pH fosters the growth of aciduric bacteria, creating an environment where acidogenic bacteria thrive, ultimately compromising the oral protective bacterial milieu(8). Thus,thesechanges in their oral environment, increase the chances of developing dental caries among diabetics.

Dental caries, a widespread chronic condition affecting individuals of all age groups, especially prevalent among children in developing countries, is characterized by the degradation of mineralisedenamel caused by lactic acid from bacteria fermenting carbohydrates found in residual dietary particles within the oral cavity(13, 14). This process is initiated by acidogenic plaque flora and is exacerbated by low salivary flow, resulting in slow clearance, inadequate buffering, and reduced calcium supply for the repair of affected dental tissues(15). Two main groups of cariogenic bacteria are*Streptococcusmutans* initiating dental caries and*Lactobacilli*species that are active during disease progression (16). Dental caries are influenced by various factors, including physical, biological, environmental, behavioural, and lifestyle-related aspects. Prominent risk factors encompass a high presence of microorganisms causing caries, reduced salivary flow, suboptimal oral hygiene practices, increased sugar intake, inadequate fluoride exposure and lower socio-economic status(1).There is ambiguity regarding type 1 or type 2 diabetic subjects andthe occurrence of dental caries in comparison with healthy controls asoasme studies have reported similar, lower, and higher, but some studies did not classify subjects concerning the DM type or type of caries(17, 18).Few studies have shown that diabetic patients might be at high risk of developing caries due to more frequent meals, an increase in intake of carbohydrates, and increased levels of salivary glucose(10, 19).However, in a systematic review conducted on diabetes and caries, the authors concluded that there is evidence lacking to establish a relationship betweencaries with diabetes recommending further investigations(20).Also, across-sectional studythat examined uncontrolled blood sugar among the DM2 groupwas found to exhibit higher mean buffering capacity, than other groups suggesting a potential link between altered salivary composition in diabetes and its impact on oral health(21)(22). As both diabetes and dental caries are on an upward trend, there is a growing need to explore the influence on oral health by the salivary changes that occur in diabetes. Also, none of the research that has already been conducted has scrutinised the effects of dental caries on diabetics in the present geographical location. Hence, the present investigation aims to bridge this knowledge gap and emphasize the need to examine salivary changes as well as the prevalence of dental cariesand its association with bacterial counts and socio-demographic factors among patients with DM2.

Material and methods

Source of data

A cross-sectional study was conducted among 100 subjects, 50 with Type 2diabetes and 50 healthy individuals matched for sex. Individuals with fasting blood glucose levels of over 120 mg/dl for a minimum of 2 years were considered diabetic. Those with type I or gestational diabetes were excluded along with those with a history of smoking or recent antibiotic therapy.

The sample size was estimated to be 45 which was rounded off to 50 in each group. This calculation was performed using G Power version 3.1.9.7 (Heinrich-Heine-University, Dusseldorf, Germany) program with allocation ratio at 1:1, effect size fixed at 0.5, power of the study at 80% and alpha error at 0.05.

In this study, stratified random sampling, was employed to ensure balanced representation across key variables. The total population was divided into four distinct strata based on health status (Type 2 Diabetes and non-diabetic) and sex (male and female). From each stratum, 25 individuals were randomly selected: 25 diabetic males, 25 diabetic females, 25 non-diabetic males, and 25 non-diabetic femalesfrom the OPD of a medical institution. This approach ensured that both sexes were equally represented (50% male and 50% female) and that each health status group contributed equally to the sample (50 individuals with Type 2 Diabetes and 50 without). The study was conducted between November 2023 to January 2024.

Method of Data Collection

Socio-demographic and medical information

Firstly, demographic data was gathered through a self-designed questionnaire administered to participants, which included details such as age, sex, socioeconomic status, dietary habits, oral hygiene practices, and specifics of the last dental visit. This information was collected through an interview format.Following the questionnaire, the details of their medical history were obtained from the patient's case sheet.

Clinical examination

A clinical examination was conducted to assess the presence of dental caries according to the DMFT (Decayed, Missing, Filled Teeth) index following the WHO criteria. The patient was examined while seated on a chair using a CPI probe and plain mouth mirror in natural light. (Figure 1a).The presence of root caries was recorded as present or absent. Training for the examiner in identifying dental caries was done by two experts from the Department of Public Health Dentistry. Scoring was practised on ten subjects who were recalled after 7-10 days. Intra-rater kappa for coronal and root caries were 0.86 and 0.72 respectively.

Estimation of pH

The salivary pHwas recordedwith the aid of pH strips placed under the tongue for a few seconds, making sure it was fully saturated with saliva. The strip was quickly removed and the alteration in colour was compared with the colour chart provided by the manufacturer. The change in the colour of the strip was recorded to indicate pH. Colour change to red or pink indicated a very acidic pH of < 4, orange or yellow signified a moderately acidic pH (around pH 4-6) and the colour green represented a neutral pH of 7 (Figure 1b).

Collection of saliva

Collection of saliva was doneon a fasting state in the morning (when the patient reported for blood sugar examinations) considering the effects of circadian rhythm. The patient was asked to rinse their mouth with plain water to remove food remnants and wait for 5 minutes (oral examination was done) before saliva collection to prevent dilution. The patient was seated in a coach-man position and asked to drool the saliva collected on the floor of the mouth into a sterile container. Saliva once collected was stored with cold packs and transferred to the laboratory for microbial analysis on the same day. The containers with salivary samples were coded with the initials of the patient along with sex. Hence the identities were masked and the technician was unaware of the presence of diabetes (Figure 1c).

Microbial analysis

The microbial analysis involved estimating Streptococcus Mutans and Lactobacillus acidophilus, whichwere cultured from the collected salivary samples. The samples underwent serial dilution with a sterile diluent (saline solution) and were then cultured for the microorganisms of interest using selective agar plates while inhibiting the growth of non-target bacteria. *S.mutans*, was cultured on mitis salivarius agar supplemented with bacitracin and sucrose whereas, for *L. acidophilus*, Rogosa SL agar was used. The plates were then incubatedfor 72 hours at 37 °C. The number of colonies was counted and expressed as colony-forming units per millilitre (CFU/mL) (Figure 2).

Ethical Clearance

Participants from both groups were explained the intention and implications of the study allowing individuals to make an autonomous decision about their involvement. Additionally, assurance regarding confidentiality of their personal information was guaranteed before informed consent was obtained. Clearance from the Institutional Ethical Board was obtained before data collection.(IEC-IDS/IDS/SOA/2023/1-44)

Data Analysis

The data collected was transferred to MS Excel (Version 16.16.27 (201012)) on the same day. Descriptive and inferential statistics were conducted using SPSS Version 23 (IBM, Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test, and descriptive statistics were presented using frequencies & percentages and means& standard deviations. The Chi-square test was employed to evaluate the association between blood sugar levels and demographic variables, while the Mann-Whitney U test was utilized for quantitative variables. Stepwise backward linear regression analysis was conducted to examine the impact of each variable on caries. The significance level was set at $p < 0.05$.

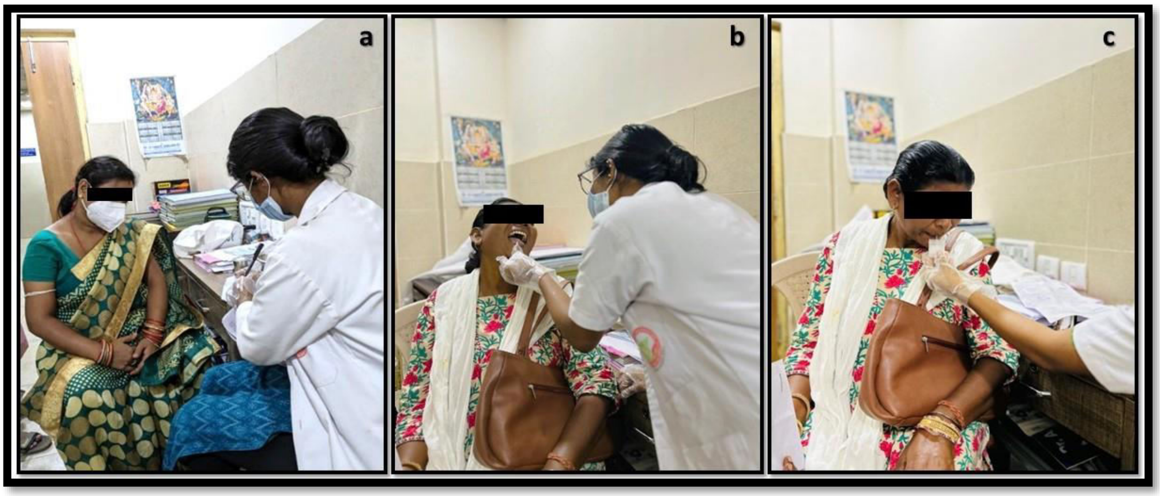

Figure 1a. Collection of paitent data; **1b.** Estimation of salivarypH; **1c.** Collection of saliva sample

Figure 2. Bacterial culture

Results

The analyzed sample comprised a total of 100 subjects, with 50 individuals diagnosed with Type 2 Diabetes and 50 without matched according to sex. The socio-demographic details based on blood sugar levels have been described in Table 1. The majority of the study population belonged to the age group of 40-45 years especially among diabetics. In terms of educational background, the majority of patients (38%) possessed an intermediate level of education, while 20% had received professional education. A portion of the study population (16%) fell into the low educational level category, including 6% who were illiterate. Additionally, 50% of the participants were semi-professional workers. Dental attendance was better among diabetics with (74%) of them visiting the dentist in the last 6 months. Brushing once a day was the prevalent frequency in both the groups while brushing twice was higher among diabetics. While the prevalence of coronal caries (26% vs 18%) and root caries (18% vs 6%) were higher among diabetics, this was not statistically significant. (Table 1)

Table 1. Distribution of the study sample according to socio-demographic variables and its relationship with blood sugar

Socio-demographic variables		Diabetic	Non-diabetic	Total	p-value
Sex	Male	27 (54%)	26 (52%)	53(53.0%)	0.5
	Female	23 (46%)	24 (48%)	47(47.0%)	
Age	40-45 yrs	33 (66%)	16 (32%)	49 (49%)	0.001*
	46-50 yrs	6 (12%)	6 (12%)	12 (12%)	
	51-55 yrs	3 (6%)	10 (20%)	13 (13%)	
	56-60 yrs	2 (4%)	11 (22%)	13 (13%)	
	61-65 yrs	1 (2%)	5 (10%)	6 (6%)	
	> 65 yrs	5 (10)	2 (4%)	7 (7%)	
Education	Illiterate	3 (6%)	3 (6%)	6(6.0%)	0.22
	Primary school	2 (4%)	1(2%)	3(3.0%)	
	Middle school	2 (4%)	5 (10%)	7(7.0%)	
	High school	4 (8%)	6 (12%)	10(10.0%)	
	intermediate	25 (50%)	13 (26%)	38(38.0%)	
	Graduate	5 (10%)	11 (22%)	16(16.0%)	
	Professional	9 (18%)	11 (22%)	20(20.0%)	
Occupation	Unemployment	5 (10%)	13 (26%)	18(18.0%)	0.169
	Unskilled worker	0	1 (2%)	1(1.0%)	
	Semiskilled worker	2 (4%)	0	2(2.0%)	
	Skilled worker	3 (6%)	3 (6%)	6(6.0%)	
	Clerical worker	3 (6%)	3 (6%)	6(6.0%)	
	Semi-professional	30 (60%)	20 (40%)	50(50.0%)	
	Professional	7 (14%)	10 (20%)	17(17.0%)	
Dental Visit	No	13 (26%)	39 (78%)	52 (52%)	0.5
	Yes	37 (74%)	11(22%)	48 (48%)	
Frequency of tooth brushing	Once	38 (76%)	42 (84%)	80(80.%)	0.22
	Twice	12 (24%)	8 (16%)	20(20.0%)	
Dental caries	Absent	37 (74%)	41 (82%)	78(78.0%)	0.235
	Present	13 (26%)	9 (18%)	22(22.0%)	
Root caries	Absent	41 (82%)	47 (94%)	88(88.0%)	0.061
	Present	9 (18%)	3 (6%)	12(12%)	

*p < 0.05; chi square test

The prevalence of coronal and root caries was found to be 0.46±0.98 and 0.14±0.403 respectively indicating a relatively low incidence of decayed teeth in the studied population. However, root caries was found to be higher among diabetics (table 2). The mean salivary pH was 6.48±0.55, which is within the neutral range observed suggesting a balanced environment in the oral cavity, stable enough to maintain oral health and prevent acidic conditions leading to tooth decay. No differences were found with respect to a number of teeth affected with coronal caries, salivary pH, *S.mutans* or *L.acidophilus*among the two groups.

Table 2.Clinical and microbiological parameters among the study groups

Variables	Diabetic Mean (SD)	Non-diabetic Mean (SD)	Total Mean (SD)	p-value
No. of coronal caries	0.58 (1.5)	0.34 (2.9)	0.46 (0.989)	0.312
No. of root caries	0.2 (0.5)	0.08 (2.7)	0.14 (0.403)	0.025*
Salivary pH	6.42 (2.6)	6.54 (1)	6.48 (0.559)	0.377
S.mutans (CFU/ml)	56.26 (2.619)	50.72 (119.20)	53.49 (2.619)	0.950
L.acidophilus (CFU/ml)	42.78 (82.09)	36.34 (115.17)	39.56 (82.09)	0.724

*p < 0.05; Mann Whitney U test

To build models that could predict dental caries or root caries, backward logistic regression analyses were applied to the variables (Table 3). These variables included age, sex, blood sugar, occupation, education and frequency of tooth brushing. In the final regressionmodels, age was a significant predictor of dental caries and blood sugar and education for root caries.

Table 3. Association of dental caries with blood sugar and other variables

Variables	Coronal Caries		Root Caries	
	OR	p-value	OR	p-value
Age	1.552	0.005*	1.310	0.184
Sex	2.493	0.085	2.642	0.055
Blood sugar	0.402	0.098	0.337	0.039*
Occupation	1.029	0.768	1.595	0.113
Education	0.806	0.448	0.463	0.028*
Frequency of tooth brushing	1.594	0.475	0.370	0.383

*p < 0.05; OR – Odd's Ratio

Table 4 outlines the stepwise backward linear regression analysis conducted on several variables. In the final model, it was identified that blood sugar, among other variables, served as a significant predictor for the number of root caries lesions which was statistically significant. For the *S.mutans* and *L.acidophilus* colony count, frequency of tooth brushing was the final predictor from the model but was not statistically significantly associated.

Table No.4 Association clinical and microbial factors with variables

	No. of coronal caries		**No. of Root caries lesions**		**Salivary pH**		***S.mutans***		***L.acidophilus***	
	Regression coefficient	p-value	Regression coefficient	p-value	Regression coefficient	p-value	Regression coefficient	p-value	Regression coefficient	p-value
Age	0.226	0.001*	0.028	0.267	-0.029	0.443	-0.358	0.943	-0.577	0.903
Sex	0.308	0.095	0.137	0.089	-0.246	0.073	-1.268	0.933	-0.544	0.097
Blood sugar	-0.436	0.053	-0.123	0.024*	-0.175	0.114	8.205	0.577	8.736	0.532
Occupation	0.005	0.936	0.007	0.642	-0.083	0.014*	0.185	0.163	3.448	0.164
Education	-0.022	0.733	-0.032	0.464	0.103	0.068	-8.834	0.236	-7.090	0.317
Frequency of tooth brushing	0.009	0.970	-0.044	0.670	-0.033	0.816	--34.113	0.059	-29.888	0.102

*p < 0.05

Discussion:

The objective of this study was to assess dental caries, salivary pH, and the counts of S. mutans and L. acidophilus among individuals with type 2 diabetes mellitus (DM2) compared to those without. While previous research has investigated the relationship between blood sugar levels in diabetics and dental caries, our study delved into the interplay of variables known to be directly or indirectly linked to either blood glucose levels or the development of dental caries. Saliva is recognized as a critical factor in the development of dental caries. In individuals with diabetes, salivary pH undergoes alterations, creating an environment conducive to the proliferation of cariogenic microorganisms, primarily *S. mutans*, which initiate caries formation, followed by *L. acidophilus*. Nevertheless, the influence of additional socio-demographic factors on dental caries occurrence must not be disregarded and warrants consideration.

Socio-demographic variables

The mean age of the subjects was 49.94±2.64 years which was relatively younger than those included by Bangash et al.(4)(57.63 years) and Almusawi MA et al(16)(54.6 years) but comparable to that of Prathibha et al(25)at 46.5 years. The majority of the participants and diabetics were aged 40-44 years, and this was found to affect both the prevalence and number of teeth affected with dental caries but there were no other studies available with which this finding could be compared and discussed.Most of the study population were males in agreement with the findings of studies by Almusawi MA et al.(16), Bangash et al(4) and Latti et al(28). However,sexwas found to influence only salivary pH and not dental caries.

Dental caries

The overall prevalence of dental caries was 26% among those with diabetes in comparison to 18% in controls though not significant, it was in line with that reported by Bangash et al(4) but lower than those reported by Malvania EA et al(19) and Shiferaw et al(29). The mean number of teeth affected (0.58±1.5) with coronal caries in our study was found to be lesser than those described by Hintao et al(23)Similar levels of dental caries across both the groups could correspond to no significant differences observed between the groups concerning risk factors implicated in the development of cavities, such as salivary pH or microorganism presence.Thiswas similar to Hintao et al(23) but in contrast to the findings reported by many studies including those conducted by Jawed M et al(15), Latti et al(28), Malvania EA et al(19), Seethalakshmi et al(26) and Singh et al(27).Root caries was found to be low with 0.2±0.5 which contrasted with the only other study that had evaluated the presence of root caries among diabetics (23). This difference can be attributed to the wide age range of the current study population from 40 years to over 70 years and only 26% of the study participants aged over 55 years of age. Nevertheless, individuals with diabetes experienced a greater impactofroot caries, consistent with findings reported by Hintao et al(23).

Microbial Counts

The primary pathogenic oral microbes associated with the development of dental caries are S. mutans, which initiate the decrease in salivary pH conducive to the growth of *L. acidophilus*. Consequently, both of these oral pathogens were assessed in the study, but no significant differences were found between the two groups. This was different from the observations of previous studies.(15, 23, 26, 27). A precise explanation for this observation could have been provided if the study had directly measured specific blood sugar levels instead of relying on a threshold to identify diabetics(30). Even though levels of both microorganisms were found to be negatively impacted by tooth brushing frequency, the difference was not statistically significant.

Salivary pH

Salivary pH often stands as a crucial factor in the causation of dental caries. However, the reduction in pH often stems from the acid production of primary colonizers, such as *S. mutans,* which were observed to be less abundant in the study population. This explains the insignificant decline in salivary pH at 6.42 ± 2.6 even among those with DM2. Higher pH values were observed by Hintao et al.(23) and Prathibha et al(25).However, lower values were reported by Jawed M et al.(15) and Singh et al(27).

Strength and limitations

While our study comprehensively examined many factors regarded traditionally as risk factors for dental caries, it omitted considerations of factors such as the duration of diabetes, prescribed medications, additional salivary attributes, and specific blood sugar levels. The finding that diabetics are more vulnerable to root caries is statistically supported. However, the impact of other factors (e.g., oral hygiene habits, diet, medication use, periodontal diseases) was not assessed.These aspects warrant exploration in future studies to provide a more comprehensive understanding.In this study, salivary pH was assessed using commercially available pH indicator strips. While these strips provide a convenient and non-invasive method for estimating pH, they offer only approximate values based on colorimetric changes and are subject to observer interpretation.

Conclusion:

The research highlighted thatindividuals with DM2 showed increased vulnerability to root caries, a susceptibility influenced by both blood sugar levels and education. However, there were no significant differences observed between the two groups regarding coronal caries, salivary pH, *S. mutans*, or *L. acidophilus*. Notably, dental caries in the study population were found to be influenced by age, while salivary pH was influenced by sex. Although the study employed a rigorous methodology, it failed to document precise blood sugar levels, comprehensive salivary parameters, and the potential influence of medications. Longitudinal studies with larger cohorts, specifically addressing these factors among diabetics, could offer additional insights. The increased susceptibility to caries among diabetics emphasizes the importance of promoting awareness about the necessity of regular oral health check-ups for individuals with elevated blood sugar levels.

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Ethics statement: Studies involving humans have been performed with the approval of an institutional ethics committee (IEC-IDS/IDS/SOA/2023/1-44).

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