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## Original Research Article

## Salivary dynamics: Assessing pH, glucose levels, and oral microflora in diabetic and non-diabetic individual

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## ABSTRACT

**Background:** Diabetes significantly impacts oral health, including alterations in the oral microflora. Increased glucose levels in the oral cavity create an environment conducive to the growth of certain pathogenic bacteria, leading to various oral and systemic diseases. These organisms can then move to the bloodstream, causing systemic inflammation and leading to health problems like cardiovascular disease and systemic infections.

**Aim and Objective:** This study aimed to compare the salivary pH, glucose concentration, and oral microflora in diabetics and non-diabetics and to correlate that elevated salivary glucose can lead to oral and systemic infections.

**Materials and Methods:** This single-centre, cross-sectional study involved 25 diabetics and 25 non-diabetics aged 18 years and above. The study duration was 60 days. Saliva samples collected from patients of both groups were subjected to biochemical and microbial analysis. In biochemical analysis, the pH and glucose concentrations were monitored. In microbial analysis, routine and PCR-based assays were used to identify the significant organisms from the saliva samples from both groups.

**Results:** The average pH (7.028) of the non-diabetic individuals was higher, towards the neutral range; however, in diabetic individuals, the range (6.016) was towards acidic. The salivary glucose estimation results showed that the average glucose concentration in the non-diabetic group was less than in the diabetic group. The oral microbiological profile of diabetic patients differed from those of non-diabetics, where the number of bacterial colonies was significantly higher in diabetic individuals. Notably, the number of dental caries-causing bacteria *Streptococcus mutans* was 25 in diabetic and 23 in nondiabetic patients. This can be attributed to the reduced pH of the saliva and the elevated glucose level.

**Conclusion:** The oral microbiological profile of diabetic patients differed from that of non-diabetics, with diabetics having considerably more bacterial colonies. Results suggested that diabetic individuals are at a higher risk of oral and systemic infections. Hence, maintaining balanced glucose levels can help protect oral health by limiting the nutrients available for pathogenic bacteria, reducing inflammation, and supporting immune function.

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## 1. Introduction

Diabetes is a chronic metabolic disorder characterized by increased glucose (hyperglycemia) because of the pancreas's abnormal insulin secretion and action. There are two primary types of diabetes, namely, type 1 diabetes and type 2 diabetes, which come from different causes. In type 1 diabetes, the pancreas fails to secrete insulin because the body's immune system attacks the islet cells in the pancreas that make insulin. In type 2 diabetes, the pancreas produces less insulin, and the body becomes resistant to insulin. In both conditions, diabetes significantly hampers the oral health.<sup>1,2</sup> Some of the significant problems are dry mouth or xerostomia, where there is a decrease in the salivary flow, making the saliva stringy in appearance, and the flushing action is altered. Hence, there is an escalated risk of microbial accumulation leading to dental caries. Secondly, there is a rise in colonization of bacteria in the oral cavity, primarily seen in the gingiva and periodontium, causing inflammation and leading to gingivitis and periodontitis, which leads to bleeding of gums, halitosis, bone loss and mobility of the teeth.<sup>3,4</sup> Further, an increase in the sugar level creates an optimum favourable condition for opportunistic fungal infections like candidiasis, which leads to oral thrush and leukoplakia. Again, diabetes leads to vascular changes, and impaired immune functions leading to ulceration and delayed wound healing. Also, there is a change in taste perception, which is dysgeusia, because of reduced salivary flow and vascular changes. The patients also have complaints of burning sensation in mouth and they are highly intolerant to spices because of diabetic neuropathy.<sup>5,6</sup>

Monitoring salivary glucose levels provides insights into the glycemic control of diabetic patients. Elevated salivary glucose levels can increase the risk of dental caries and oral infections. Changes in salivary pH and buffering capacity may affect the oral microenvironment and influence the growth of specific bacteria.<sup>7</sup> Understanding the differences in oral microflora between diabetic and non-diabetic patients requires a comprehensive approach, considering various clinical, microbiological, and systemic factors.<sup>8</sup> Hence, this study aimed to evaluate the difference in salivary pH, glucose concentration, and oral microflora between diabetic and non-diabetic patients attending a dental hospital.

## 2. Materials and Methods

### 2.1. Study design, settings, and sample collection

This study was a single-centre cross-sectional study conducted in an eastern India tertiary care dental hospital. The study duration was 2 months (1<sup>st</sup> February 2024– 31<sup>st</sup> March 2024). The primary inclusion criteria for Group A

were diabetic patients above 18 years old with complaints of oral infections, and Group B were healthy individuals with no diabetes and complaints of oral infections. Diabetic patients included in the study had a minimum of 5 years of history with diabetes. The upper age limit was not fixed in order to achieve the sample size. The sample size was decided based on the literature survey and the frequency of incoming patients. Prior consent was taken from all the patients who participated in the study before collecting their saliva samples. An aliquot of 5 ml of unstimulated saliva samples was collected from the oral cavity of 50 patients divided into two groups (25 non-diabetic patients and 25 diabetic patients) using routine procedures. The samples were processed in the central research laboratory of the dental hospital.

### 2.2. Determination of pH of saliva sample

The pH of the saliva samples was immediately measured with the help of a pH meter within 30 minutes of the collection.<sup>9</sup>

### 2.3. Salivary glucose estimation

The estimation of salivary glucose was made using the anthrone technique. The anthrone method works based on the colour change of the reaction product between anthrone and reducing sugars. A spectrophotometer and a colourimetric technique were used to estimate the total concentrations of glucose samples collected from diabetic and non-diabetic patients.<sup>10</sup>

### 2.4. Microbiological analysis

1 mL of saliva collected from each sample was homogenized with a vortex mixer. Further, phosphate buffer saline was used to dilute it. Processed saliva samples were plated on Blood agar to isolate bacterial colonies. After that, the plates were incubated for 24 to 36 hours at 37°C. The colonies were identified using routine microbiological procedures, including gram staining and biochemical tests.<sup>11</sup>

### 2.5. Molecular identification of the bacteria

The identified axenic bacteria colonies from both groups were cultured on LB agar with an overnight incubation. DNA was isolated using a DNA isolation Kit (Hi-Media) through agarose gel electrophoresis. 16S rDNA amplified with universal primer pairs 27F, 1525R (A); 27F, 1492R (B) and 530F, 1525R (C). Amplified genes were cloned, sequenced, and identified by comparison with 16S rRNA databases. Duplicate experiments were run for each specimen. PCR amplification was performed in a thermal cycle.<sup>12</sup>

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### 3. Results

A total of 50 patients were categorized into the diabetic and non-diabetic groups based on their diabetic status. Each group consisted of 25 patients. Optimal glucose concentrations in saliva range from 0.5 to 1.00 mg/ml and do not significantly impact oral health or promote microbial proliferation. The salivary glucose estimation results showed that the average glucose concentration in the non-diabetic group was 0.71 mg/ml with a standard deviation of 0.156 (Table 1). This result is within the normal range of glucose concentration in healthy people. However, in the diabetic group, the average glucose concentration was 1.714 mg/ml with a standard deviation of 0.396, which is higher than the normal range.

From the isolation and identification of the bacteria from the saliva samples, it was evident that the patients in the diabetic group had the colonization of more bacteria compared to the non-diabetic group (Table 2). The significant bacteria isolated were *Enterococcus spp.*, *Actinobacillus actinomycetemcomitans*, *Streptococcus mutans*, *Lactobacillus spp.*, *Streptococcus anginosus*, *Streptococcus salivarius*, *Streptococcus gordonii* and *Streptococcus pyogenes*.

The highest prevalence difference values were *S. pyogenes*: 56% difference (100% vs 44% in diabetic vs non-diabetic), *S. salivarius*: 28% difference (100% vs 72% in diabetic vs non-diabetic) and *Enterococcus spp.*: 36% difference (68% vs 32% in diabetic vs non-diabetic). There was equal prevalence seen in *Lactobacillus spp.*: 100% prevalence in both groups and the PCR confirmation rate was also 100% for both groups comparison of high prevalence in both groups was seen to be *S. mutans*: 100% in diabetic vs 92% in non-diabetic and PCR confirmation rates were high for both groups (100% and 95.7%, respectively). The inference of the PCR confirmation rates showed. In the diabetic group, the highest was 100% for *S. mutans* and *Lactobacillus spp.* and the lowest was 84.2% for *A. actinomycetemcomitans*. In the non-diabetic group, the highest was 100% for *Lactobacillus spp.* and *S. pyogenes*, and the lowest was 75% for *Enterococcus spp.* Most marked differences were observed in *S. pyogenes* (56% difference), *Enterococcus spp.* (36% difference), *S. salivarius* (28% difference) and *S. gordonii* (24% difference) (Table 3).

### 4. Discussion

Diabetic patients showed consistently higher bacterial colonization across most species. The average number of patients with bacterial presence was significantly higher in the diabetic group. Both CFU counts and PCR confirmation showed similar trends. In species-specific findings, the most significant differences were observed in *S. pyogenes* (25 vs 11 patients in CFU), *S. salivarius* (25 vs 18 patients in CFU) and *Enterococcus spp.* (17 vs eight patients in

CFU). The average CFU detection in the diabetic group was 22.8 patients, whereas the average CFU detection in the non-diabetic group was 16.6 patients. Similarly, the average PCR confirmation in the diabetic group was 21.1 patients, whereas, the average PCR confirmation in the non-diabetic group was 15.4 patients. The notable pattern was in *Lactobacillus spp.*, which showed equal presence in both groups (25 patients each). *S. mutans* showed a slightly high prevalence in diabetic groups. The most significant disparities were seen in opportunistic pathogens. This study demonstrates that diabetes directly impacts the oral microbiota and the patient's overall oral health. The cleaning capacity of saliva is diminished due to an anomaly in the saliva flow rate and salivary buffering capacity. The pH of the saliva was slightly acidic due to sugar fermentation in the oral cavity, which resulted in cariogenic activities, dysbiosis, and the accumulation of more pathogenic organisms. Numerous studies have been undertaken on a similar problem, detailed in the following paragraphs.

Montevocchi et al. concluded that individuals with diabetes might experience alterations in the abundance and diversity of oral bacteria. The changes in oral microbiota composition can contribute to an environment conducive to developing oral diseases. Their study showed an increased prevalence of periodontal pathogens, such as *Porphyromonas gingivalis* and *Tannerella forsythia*, in individuals with diabetes.<sup>13</sup> These bacteria are commonly associated with periodontal disease. Conversely, there may be a decrease in the levels of beneficial bacteria, including those with anti-inflammatory properties. This imbalance can contribute to the progression of periodontal disease and compromise oral health. Saliva is crucial in maintaining oral health by controlling bacterial growth and aiding digestion. Disruptions in salivary microbiota can impact oral health. Individuals with diabetes may be more susceptible to oral candidiasis, an infection caused by the fungus *Candida sp.* Diabetes-related changes in the oral environment, such as elevated glucose levels, can promote the growth of *Candida species*, as demonstrated in a study by Mohammadi et al. and Pradhan et al., respectively.<sup>14,15</sup> Diabetes can compromise the immune response, making individuals more susceptible to infections. This impairment can affect the ability of the immune system to control and regulate the oral microbiota. Diabetic individuals may exhibit increased biofilm formation on oral surfaces. Biofilms are communities of microorganisms embedded in a protective matrix, and their presence can contribute to the development of dental plaque and other oral health issues. Oral health is interconnected with systemic health. The changes in the oral microbiota associated with diabetes may contribute to systemic inflammation, potentially influencing the overall health of individuals with diabetes.<sup>16</sup> In a study involving patients with periodontitis and type 2 diabetes, there

**Table 1:** Salivary glucose concentration in saliva samples of both groups

Diabetic group	pH	Sugar concentration mg/ml	Non-Diabetic group	pH	Sugar concentration mg/ml
DB 1	6.0	1.5	ND 1	6.9	0.5
DB 2	6.1	1.5	ND 2	7.0	0.7
DB 3	5.9	1.25	ND 3	7.2	0.9
DB 4	5.8	2.5	ND 4	7.1	1
DB 5	6.2	1.75	ND 5	7.0	0.55
DB 6	6.0	1.85	ND 6	6.8	0.75
DB 7	6.1	2.5	ND 7	7.2	0.6
DB 8	5.8	1.65	ND 8	7.2	0.85
DB 9	6.2	1.5	ND 9	7.0	0.5
DB 10	6.1	1.75	ND 10	6.8	0.85
DB 11	5.9	1.4	ND 11	7.2	0.9
DB 12	6.0	1.85	ND 12	7.0	0.55
DB 13	5.9	1.6	ND 13	7.1	0.75
DB 14	6.0	1.25	ND 14	6.9	0.7
DB 15	6.2	1.1	ND 15	6.8	0.8
DB 16	6.1	1.95	ND 16	7.2	0.5
DB 17	6.0	2.4	ND 17	7.0	0.7
DB 18	5.8	1.65	ND 18	7.1	0.95
DB 19	6.2	1.5	ND 19	6.8	0.6
DB 20	6.2	2.5	ND 20	7.2	0.75
DB 21	6.1	1.65	ND 21	7.1	0.55
DB 22	5.9	1.35	ND 22	6.9	0.6
DB 23	6.0	1.65	ND 23	7.0	0.75
DB 24	5.9	1.4	ND 24	7.0	0.5
DB 25	6.0	1.85	ND 25	7.2	0.95
Mean	6.016	1.714	Mean	7.028	0.71
Std deviation	0.128	0.128	Std deviation	0.140	0.156

**Table 2:** Bacterial species identified from the saliva samples in both groups.

Group	Bacterial species identified	Number of patients harbouring the bacteria with a minimum of 25 CFU/ml	Confirmation of Bacteria Using PCR
Diabetic group	<i>Enterococcus spp.</i>	17	15
	<i>Actinobacillus</i>	19	16
	<i>actinomycetemcomitans</i>		
	<i>Streptococcus mutans</i>	25	25
	<i>Lactobacillus spp</i>	25	25
	<i>Streptococcus anginosus</i>	25	23
	<i>Streptococcus salivarius</i>	25	22
	<i>Streptococcus gordonii</i>	21	19
	<i>Streptococcus pyogenes</i>	25	24
	<i>Enterococcus spp.</i>	8	6
	<i>Actinobacillus</i>	12	10
	<i>actinomycetemcomitans</i>		
Non-Diabetic group	<i>Streptococcus mutans</i>	23	22
	<i>Lactobacillus spp</i>	25	25
	<i>Streptococcus anginosus</i>	21	20
	<i>Streptococcus salivarius</i>	18	15
	<i>Streptococcus gordonii</i>	15	14
	<i>Streptococcus pyogenes</i>	11	11

**Table 3:** Bacterial prevalence analysis

Bacterial Species	Diabetic Prevalence (%)	Non-diabetic Prevalence (%)	Difference (%)	PCR Confirmation Rate Diabetic (%)	PCR Confirmation Rate Non-diabetic (%)
<i>Enterococcus spp.</i>	68.0%	32.0%	36.0%	88.2%	75.0%
<i>A. actinomycetemcomitans</i>	76.0%	48.0%	28.0%	84.2%	83.3%
<i>S. mutans</i>	100.0%	92.0%	8.0%	100.0%	95.7%
<i>Lactobacillus spp.</i>	100.0%	100.0%	0.0%	100.0%	100.0%
<i>S. anginosus</i>	100.0%	84.0%	16.0%	92.0%	95.2%
<i>S. salivarius</i>	100.0%	72.0%	28.0%	88.0%	83.3%
<i>S. gordonii</i>	84.0%	60.0%	24.0%	90.5%	93.3%
<i>S. pyogenes</i>	100.0%	44.0%	56.0%	96.0%	100.0%

was a significant decrease in the occurrence of a few bacteria after the diabetic treatment. Like our study, they also concluded that bacteria can be used as microbial biomarkers, which can indicate oral and other systemic diseases related to diabetes.<sup>17</sup> In a study from Uttar Pradesh, India, it was demonstrated that organisms like *Lactobacillus*, *Streptococcus*, and *Porphyromonas* were significantly higher in diabetic patients, and they had a higher risk of caries, gingivitis, and periodontitis when compared to non-diabetic participants.<sup>18</sup> Rawal et al. concluded that 80% of diabetic patients had significant oral health problems and suggested that oral health rehabilitation programmes should be integrated with diabetic health control programmes.<sup>19</sup> In another study from Gujarat, India, it was established that diabetes increases the chances of dental caries as a higher amount of *S. mutans* colonies were isolated from diabetic patients in their study.<sup>20</sup> Similar results were obtained in our study, too.

Research has suggested a correlation between salivary glucose levels and blood glucose levels. A study from China established that, in individuals with type 2 diabetes, there were variations and linkages in the flora composition at the oral and intestinal exit locations of the dysbacteriosis. There may be a connection between type 2 diabetes mellitus and ectopic colonization of oral flora in the gut. Furthermore, elucidating the oral-gut-transmitting bacteria can be a crucial reference for future type 2 diabetes mellitus diagnosis and treatment.<sup>21</sup> However, the correlation between blood glucose and other body fluids like urine may be more direct and reliable than the relationship between salivary glucose and other body fluids.<sup>22</sup> There are challenges and limitations associated with measuring salivary glucose. Various factors, including diet, oral health, and the saliva flow rate, may influence salivary glucose levels. Additionally, glucose concentration in saliva tends to be lower than in blood.<sup>23</sup> Research in this area continues, and technologies for measuring salivary glucose are being developed and streamlined. Advances in biosensor

technology and analytical techniques may improve accuracy and reliability in the future.<sup>24</sup>

## 5. Conclusion

The oral microbiological profile of diabetic patients differed from those of non-diabetics, where the number of bacterial colonies was significantly higher in diabetic individuals. This can be attributed to the reduced pH of the saliva and the elevated glucose level. In such patients, tooth decay can happen much earlier than non-diabetic patients. Managing diabetes through effective blood sugar control, maintaining good oral hygiene practices, and addressing oral health issues promptly are essential strategies to prevent and manage oral health in diabetic patients.

## 6. Limitations

One of the foremost limitations was the short study duration of 2 months, due to which the sample size was restricted to 25 in each group. Secondly, we did not have the facilities for culturing anaerobic microorganisms, which could have added more value to our study.

## 7. Ethical Approval

This study was conducted after the approval by the Institutional Ethical Committee via letter no. IEC-IDS/IDS.SOA/2023/I-41 dated 10<sup>th</sup> October 2023. This article is part of larger study involving diabetic population with multiple dental problems.

## 8. Source of Funding

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## 9. Conflict of Interest

We declare no conflict of interest in the context of this research.

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