Forty-five patients were divided into three groups consisting of patients:

- Group A: Patients with type 2 DM with caries
- Group B: Patients with type 2 DM without caries
- Group C: Age-matched healthy nondiabetic individuals (control).

Saliva samples were subjected to semiautomatic salivary glucose estimation by the glucose oxidase-peroxidase method, using the Tulip glucose estimation kit. Swabs were immediately inoculated onto Mitis Salivarius Bacitracin agar and Man Rogosa Sharpe agar.

**Results:** In Group A, statistically significant positive correlation was found between *S. mutans* and salivary glucose \( r = 0.858 \) as well as *L. acidophilus* and salivary glucose \( r = 0.853 \). In Group B, a statistically significant positive correlation was found only between *S. mutans* and salivary glucose \( r = 0.705 \) and not between *L. acidophilus* and salivary glucose \( r = 0.387 \). The control group did not show a statistically significant correlation.

**Conclusion:** It is established that salivary glucose levels reflect the diabetic state of an individual. The salivary glucose level predicted a 1.7 times higher caries susceptibility in a diabetic, as shown by results in this study. Salivary glucose causes an increase in the cariogenic load in diabetic patients, thus warranting a modification of the Keyes triad.

**Keywords:** Decayed, Missing, and Filled Teeth, diabetes mellitus, type 2, Lactobacillus acidophilus, Oral Hygiene Index, saliva, Streptococcus mutans

**Introduction**

The prevalence of type 2 diabetes mellitus (DM) is set to increase 4 times by 2030 with approximately 510.8 million individuals being affected worldwide by this epidemic.\(^1\) Patients with DM have been linked with an increased risk for gingivitis and periodontal disease;\(^2\) however, its impact on dental caries is not clearly explained.\(^3,4\) Increased salivary glucose levels have been positively correlated with the serum glucose levels of diabetic patients.\(^5-9\) Thus, the aim of the study is to assess if the elevated salivary glucose levels provide an environment conducive to the growth of cariogenic microorganisms specifically *Streptococcus mutans* and *Lactobacillus acidophilus*.

**Materials and Methods**

After ethical clearance (EthicalComm./GDCH/2015-1/OralPath-1), samples were procured from individual patients ranging from 40 to 60 years of age at a government medical hospital (Goa, India).

The patients diagnosed with DM in the age range of 20–75 years and the age-matched healthy individuals for control were included in the study.

The patients with any other systemic diseases and on regular medication for the same, tobacco or alcohol habits, mentally challenged individuals, pregnant women and uncooperative patients were excluded.

The present study included 45 patients who were categorized into three groups, each consisting of 15 patients:

- Group A: Patients with type 2 DM with caries
- Group B: Patients with type 2 DM without caries
- Group C: Age-matched healthy nondiabetic individuals (control).

The details and the need for the study were explained to the subjects and informed consent obtained. A detailed case history was recorded followed by a general and

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oral examination (including Oral Hygiene Index (OHI) index and decayed missing filled teeth (DMFT) score). Spit-method was used to collect 2 ml unstimulated saliva samples. Oral swabs were used to collect plaque samples from bilateral mandibular 1st molars or mandibular 2nd molars or mandibular premolars.

Saliva samples were subjected to semiautomatic salivary glucose estimation by the glucose oxidase-peroxidase method, using the Tulip glucose estimation kit. Swabs were immediately inoculated onto Mitis Salivarius Bacitracin agar and Man Rogosa Sharpe agar, which were then incubated anaerobically (Laminar Air Flow Chamber) at 37° for 48 h.

The identification of the colonies was made based on the colony morphology, forms and culture characteristics. The confirmatory tests included Grams staining and catalase test.

The data were tabulated and subjected to statistical analysis using SPSS version 20 software (SPSS Inc., Chicago, Ill., USA). The mean, standard deviation, and P value were assessed by applying ANOVA test and Pearson correlation was used for comparison of DMFT and salivary glucose. *S. mutans* and salivary glucose, *L. acidophilus* and salivary glucose in all three groups. Odd’s ratio was calculated to ascertain the risk of developing caries in individuals with type 2 diabetes mellitus.

**Results**

The mean (n) salivary glucose (Group A: n = 4.613, Group B: n = 3.547, Group C: n = 1.147), *S. mutans* count (Group A: n = 100.00, Group B: n = 49.33, Group C: n = 1.147) and *L. acidophilus* (Group A: n = 60.00, Group B: n = 25.33, Group C: n = 13.53) count were highest in Group A and lowest in Group C [Figure 1].

In Group A [Figure 2], statistically significant positive correlation was found between *S. mutans* and salivary glucose (*r* = 0.858) as well as *L. acidophilus* and salivary glucose (*r* = 0.853). In Group B, a statistically significant positive correlation was found only between *S. mutans* and salivary glucose (*r* = 0.705) and not between *L. acidophilus* and salivary glucose (*r* = 0.387). The control group did not show a statistically significant correlation.

Within Group A [Figure 3], the DMFT score increased with increasing value of salivary glucose (*r* = 0.778). The OHI score was significantly higher in Group A than Group B.

The Odd’s ratio was found to be 1.7 based on the salivary glucose levels.

**Discussion**

Dental caries is a multifactorial infectious disease affecting the dental hard tissues that is characterized by microbiome dysbiosis with an increase in the cariogenic bacteria.[10] A key factor in promoting the dysbiosis is glucose.[11] Keyes in the year 1960 proposed that three factors: host, microbiota, and substrate are essential for caries occurrence; suggesting that control of any one of the factors would be sufficient to prevent the disease. Subsequently, Newbrum added time as a factor since caries considered as a chronic disease which is clinically detectable only after significant loss of mineralized enamel.[12] Glucose or other carbohydrates promote the growth and acid production of *S. mutans* but not its adherence, since the extracellular polymers are synthesized exclusively from sucrose. Lactobacilli are aciduric and acidogenic bacteria.[13] Patients with DM have been shown to have significantly elevated salivary glucose levels.[5‑9] that may be independent of the frequency of dietary intake of carbohydrates, suggesting an upregulated link between the host and oral microbiome conferring a greater susceptibility to dental caries in this specific population.

In this study, we explored the possible relationship between diabetes and the development of caries by examining the caries potential in type 2 diabetic patients using, the following parameters: Salivary glucose, DMFT index, and *S. mutans*, *L. acidophilus* counts.

A positive correlation between salivary glucose and cariogenic load was established in this study by a statistically significant result. Similar findings were noted by, Syrjälä et al.,[3] where they suggested that poor glycemic control combined with high levels of MS and lactobacilli is particularly detrimental for oral health. Kampoo et al.[14] demonstrated similar results to the present study. In spite of the sucrose restriction, oral conditions in diabetics are favorable for the growth of cariogenic bacteria, suggesting that the leakage of blood glucose in to the oral cavity could favor the growth of the cariogenic organisms. According to the ecological plaque hypothesis, caries is considered to be a biofilm-mediated disease where a catastrophic ecological shift in the plaque triggers an imbalance in the equilibrium of the dental hard tissues that is characterized by microbiome dysbiosis with an increase in the cariogenic bacteria.[10] A
leading to demineralization and carious lesion formation.\textsuperscript{15} In a systematic review, Mascarenhas \textit{et al.}\textsuperscript{13} deduced that regardless of food debris, salivary flow rate fluctuations or presence of local neuropathy, there is a consistent increase in salivary glucose due to type 2 DM. Besides the salivary glucose level, salivary calcium, salivary flow rate, and salivary pH were altered\textsuperscript{16} driving the ecological shift.

The DMFT score was directly proportional to the salivary glucose levels in Group A and also to the OHI of diabetic patients. Latii \textit{et al.}\textsuperscript{15} reported an increase in the DMFT score of diabetics attributing it to increased blood sugar levels which caused increased SM count. Similar findings were noted by Singh \textit{et al.}\textsuperscript{16} where DMFT score of the patients with type-II diabetes was very high in comparison with healthy individuals. They also put forward that the function of neutrophils is altered with increasing levels of salivary glucose leading to accelerated microbial accumulation and thus placing diabetics at a higher risk of tooth damage. Taken together, the foregoing findings introduce a modifier to the classic Keyes triad in the etiology of dental caries, namely the host with poor glycemic control [Figure 4].

Correlating the parameters of DMFT, salivary glucose, and cariogenic bacterial load in diabetics, the following hypothetical complex is proposed:

\textbf{Diabetic oral hygiene complex = DMFT + salivary glucose + cariogenic bacterial load.}

Standardization of results obtained in this study, can be achieved by testing the hypothetical formula:

\textbf{Oral Hygiene Index} $\propto$ \textit{diabetic oral hygiene complex}

Computation of a numerical range for the diabetic oral hygiene complex can be achieved, by testing the above formula in large scale population studies.

This being a pilot study the sample size is limited, hence results based on a larger sample size need to be evaluated.

\textbf{Conclusion}

It is established that salivary glucose levels reflect the diabetic state of an individual. The salivary glucose level predicted a 1.7 (odds ratio) times higher caries susceptibility in a diabetic, as shown by results in this study. Salivary glucose causes an increase in the cariogenic load in diabetic individuals, thus warranting a modification of the Keyes triad. Computing the diabetic oral hygiene complex score can sensitize these patients to follow meticulous plaque control measures for prophylaxis against dental caries. All diabetic individuals need to be screened for the diabetic oral hygiene complex at the primary level of examination to reduce the ill effects of poor oral health in diabetics.
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Conflicts of interest
There are no conflicts of interest.

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