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Original Article

Evaluation of salivary glucose levels and oral health status in healthy individuals: An *ex vivo* study

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Abstract

According to the World Health Organization, 19.4 million people in India had diabetes in 1995, and this figure is projected to rise to 57.2 million by 2025, accounting for one-sixth of the global total. Saliva is referred to as the "mirror of the body" since it functions as a health indicator in the mouth and throughout the body. Since diabetes has been declared a pandemic of non-communicable diseases, a chair-side, non-invasive, less technique-sensitive, cost-effective approach should be considered. The study evaluated the association between salivary glucose levels and oral health status of healthy individuals. After obtaining the informed consent, an oral cavity was examined per the WHO oral health status form for adults (Annexure 1). The standard salivary glucose level was measured using the O-Toluidine reagent method using a double-beam spectrophotometer. The blood glucose levels were measured using an electronic blood glucose meter. On comparison of glucose levels according to CPI score and DMFT experience using ANOVA, a p-value of 0.08 (>0.05) and 0.95 (>0.05) was obtained, which was statistically non-significant. The bivariate correlation between Salivary Glucose Level (SGL) (ug/ml) and blood Glucose Level (BGL) (mg/dl) was checked using the Pearson correlation coefficient. There was a statistically significant moderate and positive correlation between Blood Glucose Levels (mg/dl) and salivary Glucose Levels (ug/ml). Within the limitation of the study, the study concluded that there is no significant association between salivary glucose levels and oral health status of healthy individuals. However, a positive correlation exists between salivary glucose levels and blood glucose levels in healthy individuals.

Keywords: saliva, salivary glucose levels, blood glucose levels, healthy individuals.

Abbreviations: BGL – Blood Glucose Level; CPI – Community Periodontal Index; DM – Diabetes Mellitus; DMFT – Decayed Missing Filled Teeth; SGL – Salivary Glucose Level; WHO – World Health Organisation.

Introduction

Diabetes mellitus (DM) is a metabolic syndrome characterized by disturbances in the metabolism of carbohydrates, lipids and proteins. It considers chronic hyperglycemia as the result of insulin secretion breakdown or increased cellular resistance to insulin action [1]. According to the World Health Organization, 19.4 million people in India had diabetes in 1995, and this figure is projected to rise to 57.2 million by 2025, accounting for one-sixth of the global total. By 2030, the updated estimates are 80.9 million [2].

The primary methods to diagnose DM and monitor blood glucose levels have traditionally been fasting blood glucose, a combination of fasting blood glucose plus a 2 h test after glucose loading (2 h postprandial), and an oral glucose tolerance test (OGTTs) [3]. It has been estimated that 20.5% of the population refuses any medical attention due to needle prick anxiety [4].

Saliva is important for maintaining the balance of the oral ecology. Saliva is referred to as the "mirror of the body" since it functions as a health indicator in the mouth and throughout the body [5]. It bathes and mimics the position of the bloodstream in the oral

cavity because it includes serum constituents, which are assessed in routine blood tests to track health and diseases [6]. These components come from the salivary glands' local vasculature and gingival crevicular fluid. It can be obtained non-invasively and by people with no experience, such as the patient. The fluid collection does not necessitate using any special equipment [2]. Since diabetes has been declared a pandemic of non-communicable diseases, a chair-side, non-invasive, less technique-sensitive, cost-effective approach that allows people to track their glucose levels regularly should be considered.

The study evaluated the association between salivary glucose levels and oral health status of healthy individuals.

Material and methods

After procuring Institutional ethical clearance (Cert. No: ABSM/EC/101/2021) and CTRI registration (CTRI/2021/08/035612). The study was conducted in the Department of Conservative Dentistry and Endodontics, A.B Shetty Memorial Institute of Dental Sciences, Mangaluru and Central Research Lab, KS Hegde Medical Academy, NITTE Deemed to be University, Deralakatte, Mangaluru.

The study included participants without any past medical illness between the ages of 35 and 44. Participants who were under medication that alters salivary flow and composition were under radiotherapy or were not willing to take part in the study were excluded.

Experimental method

All 29 patients were informed about the study, and the informed consent was recorded. A detailed case history as per the WHO Oral Health Assessment Form for Adults (2013) (Annexure 1) [7] was recorded, and a clinical examination of the oral cavity was done.

All 29 salivary samples of the volunteers were collected 2 hours after breakfast between 9 a.m. and 11 a.m., and volunteers were asked not to refrain from having anything in between. Volunteers were also asked to sit upright on the dental chair with their heads kept forward and instructed not to swallow, speak, or make any movements of their heads.

In order to obtain the saliva, the patient was asked to swallow the saliva in the mouth and then to remain still without moving the tongue or swallowing the saliva for one minute. 2 ml of saliva was collected in a BD VACUTAINER -2 ml sterile container by passive drool method.

Laboratory method

The salivary samples obtained were centrifuged in the laboratory centrifuge machine. The collected saliva was stored in ultra low temperature freezer at -81°C.
Preparation of protein: In a dry test tube, 3 ml of distilled water and 0.5 ml of saliva were combined thoroughly with the help of CM 101 Cyclo Mixer. A 1.5 ml of 10% Tri-chloroacetic acid (TCA) was obtained by weighing accurately with the help of Sartorius Weighing Balance, mixed completely again with CM 101 Cyclo Mixer, and set alone for 10 minutes before being filtered into a dry test tube.
Development of colour: Six test tubes were filled with GS solutions (AGAPPE) ranging from 1 to 5 µg/ml, while a seventh test tube was filled with 1 ml of protein-free filtrate. 5 ml of O-toluidine was added to each of these tubes and thoroughly mixed with CM 101 Cyclo Mixer.

The tubes were placed in a boiling water bath for 10 minutes, cooled, and the OD was measured at 620 nm using a PC-based double-beam Spectrophotometer.

Method of estimation of blood glucose level

The glucose level in the patient's blood was measured by applying a drop of blood to a chemically treated, disposable "test-strip", which is then inserted into an electronic blood glucose meter. (Accu-Chek Active Blood Glucose Glucometer Kit).

Statistical analysis

The sample size of 29 was calculated using nMaster Software of Version 2 with a standard deviation of 1.91 and margin of error 0.7 at 95% confidence level.

The chi-square test was applied to verify the existence of statistical significance between group variations, with the significance level being set at p-value ≤0.05. If statistical significance is found, a stepwise multiple comparison procedure, in the form of a Tukey test for post-hoc comparison, was done.

The normality of numerical data of the blood glucose level with salivary glucose level was checked using the Shapiro-Wilk test, and it was found that the data followed a normal curve; hence, parametric tests have been used for comparisons.

The bivariate correlation between Salivary Glucose Level (SGL) (ug/ml) and blood Glucose Level (BGL)

Table 1: Descriptive statistics of glucose levels and DMFT experience.

		Glucose levels (ug/ml)	DMFT
N		29	29
Mean		3.56	8.10
SD		0.61	3.10
Minimum		2.48	2
Maximum		4.96	14
	Q1	3.11	6
Percentiles	**Median**	3.45	8
	Q3	3.94	10.5

Table 2: Mean of Community Periodontal Index(CPI) score.

		CPI 0	CPI 1	CPI 2	CPI 3
N		29	29	29	29
Mean		3.59	0.72	1.41	0.28
SD		1.59	0.92	1.27	0.80
Minimum		2	0	0	0
Maximum		6	3	4	4
Frequency		7	0	17	5
Percent		24.1	-	58.6	17.2
	Q1	2	0	0	0
Percentiles	**Median**	3	0	1	0
	Q3	5.5	1	2	0

(mg/dl) was checked using the Pearson correlation coefficient. For the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving power to the study as 80%.

Results

The descriptive analysis of glucose levels and DMFT experience of 29 salivary samples that were collected had a mean of 3.56 ug/ml and a mean DMFT of 8.10 with a standard deviation of 0.61 ug/ml for the glucose levels and 3.10 for the DMFT scores (Table 1).

The Mean CPI score shows a mean and standard deviation of 3.59 and 1.59 for CPI0, 0.72 and 0.92 for CPI 1, 1.41 and 1.27 for CPI2, 0.28 and 0.80 for CPI 3. The CPI 2 had the highest distribution among the 29 study participants included in the study, with a percentage of 58.6% (Table 2).

On comparison of glucose levels according to CPI score using ANOVA, a p-value of 0.08 (>0.05) was obtained, which was statistically non-significant (Table 3).

On the correlation between glucose levels and DMFT experience, a p-value of 0.95(>0.05) was obtained, which was statistically non-significant (Table 4).

Test for normality between the salivary glucose levels (SGL) and blood glucose levels (BGL) shows p values of 0.961 and 0.941 (>0.05), indicating that normality was followed (Table 5).

A descriptive analysis of the data between the SGL and BGL shows a mean and standard deviation of 3.5590 and 0.61167 for SGL and 131.03 and 4.740 for BGL, respectively (Table 6).

There was a statistically highly significant moderate & positive correlation between BGL (mg/dl) & SGL Results (ug/ml) ($p<0.01$ and r value- 0.4–0.7). A positive correlation indicates that as the value of one variable increases, the other also increases (Table 7).

Discussion

Normal blood glucose level 2hrs postprandial is ≥140 mg/dl by drawing the blood from the peripheral veins is the most common method for detecting and screening diabetes [8]. Saliva is becoming more and more effective as a diagnostic aid. It's a body fluid with a unique structure and function [9]. Human saliva is a

Table 3: Comparison of glucose levels according to CPI score.

CPI highest	N	Mean	SD	Min	Max	ANOVA	
						F	P-value
CPI 0	7	4.01	0.76	3.01	4.96		
CPI 2	17	3.41	0.49	2.48	4.52	2.80	0.08 (NS)
CPI 3	5	3.43	0.57	2.87	4.18		

Note: * – $p<0.05$ statistically significant, NS – $p>0.05$ non-significant.

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Table 4: Correlation between glucose levels and DMFT experience.

	Glucose Levels *vs.* DMFT
r	0.01*
P-value	0.95 (NS)

Note: *– p<0.05 statistically significant; NS – p>0.05 non-significant.

Table 5: Test for normality between SGL and BGL.

	Shapiro-Wilk		
	Statistic	**df**	**P-value**
SGL results (ug/ml)	.961	29	.354
BGL (mg/dl)	.941	29	.107

Table 6: Descriptive statistics of data between the SGL and BGL.

	N	Range	Minimum	Maximum	Mean	Std. Error of Mean	Std. Deviation	Variance
SGL results (ug/ml)	29	2.48	2.48	4.96	3.5590	.11358	.61167	.374
BGL (mg/dl)	29	16	122	138	131.03	.880	4.740	22.463

specific secretion produced by the major and minor salivary glands that aid in the regular physiologic activities of oro biological tissues. Dawes *et al.* stressed the value of precision in saliva measurements. The presence of a circadian rhythm and fasting has been shown to affect salivary flow rate, making the test time-point important. As a result, saliva was obtained between 9:00 and 11:00 a.m. [10]. In another study, Borg A. *et al.* (1998) found that the concentration of glucose in the saliva of the parotid gland increased significantly 2 hours after glucose or food intake in individuals with diabetes mellitus compared to healthy [11].

The WHO guidelines Recommendation 5 suggests that "Glucose should be measured immediately after collection by near-patient testing, or if a blood sample is collected, plasma should be immediately separated, or the sample should be collected into a container with glycolytic inhibitors and placed in ice water until separated before analysis" [12].

Grey-top tubes that contain fluoride ions can be employed to inhibit glycolysis. Weissman M *et al.* conducted a study on the effects of inhibitors of glycolysis (fluoride and thymol) on serum glucose determinations; blood samples from over 200 patients were collected in tubes containing the glycolysis inhibitors and in tubes without these inhibitors or preservatives. They concluded that serum with the use of an antiglycolytic agent is adequate for glucose determinations unless the delay between collection and processing is minimum [13, 14]. Rapid separation is an option with our current gel-separation lithium/heparin (Li/Hep) tubes (BD Vacutainer® PST™; BD Diagnostics Preanalytical Systems), although the recommended centrifugation time is 10 min because an incomplete barrier may form with shorter periods, gel flow may be impeded at temperatures [15].

Saliva plays a critical role in preserving oral cavity homeostasis by stabilizing the environment, making it one of the indicators for effective disease treatment and risk estimation [16]. In controlled diabetics, salivary glucose levels had a very high correlation coefficient (r=0.841) and a statistically significant, meaningful relationship (p=0.001) with blood glucose levels. Kortuem and Shannon *et al.* found a rise in salivary glucose following an increase in blood glucose following a glucose load, implying comparable outcomes [17]. Normal glucose levels in saliva are 0.5–1.00 mg/100 ml [18]. The standard glucose concentration (AGAPPE) required to measure the standard glucose level of saliva is 1–5 µg/ml [2] Saliva plays an important role in oral cavity homeostasis because it stabilizes the oral cavity's environment; as a result, it serves as an excellent marker for the early detection of disease, which leads to more efficient treatment, risk assessment, glucose level assessment, and a simple, non-invasive alternative

Table 7: Bivariate correlations.

	SGL results (ug/ml) & BGL (mg/dl)
Pearson correlation r value	.625
P-value	.000
N	29

to blood and urine tests [18]. Diagnostic technology advancements have tremendous potential to achieve the long-term aim of clinically validated, saliva-based health screening and early warning tests for oral disease and other systemic conditions [19].

Salivary glucose estimation is a diagnostic tool in assessing glucose levels provided precautions is taken about the time and method of collection and estimation. However, to establish salivary glucose estimate as a diagnostic technique for determining glucose levels in DM, additional research on bigger populations, in other geographic locations, and among persons of different age groups is necessary.

Conclusion

Within the limitation of the study, the study concluded that there is no significant association between the salivary glucose levels and oral health status of healthy individuals. But there exists a positive correlation between the salivary glucose levels and blood glucose levels in healthy individuals.

Conflict of interest

The authors declare no conflict of interest.

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