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Original Research

Standardizing the collection and measurement of glucose in saliva and its relationship with blood glucose concentration

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Abstract

Background: Given that diabetes has been declared a pandemic of non-communicable diseases, a chairside, non-invasive, less technique-sensitive, cost-effective approach that reduces discomfort, anxiety, and fear among the general population and encourages them to monitor the level of glucose on a regular basis should be considered. **Methods:** All salivary samples were collected 2 hours after breakfast between 9 and 11 a.m. Volunteers were asked to sit upright on the dental chair and 2 ml of saliva were collected in the sterile fluoridated container. The standard salivary glucose level was measured using the O-toluidine reagent method using a double beam spectrophotometer. **Results:** The study found out that the salivary glucose levels are measured more accurately using the O-toluidine reagent method. A 1–5 µg/ml of glucose standard solution is used for standardizing normal salivary glucose levels using the O-toluidine reagent method and measuring the optical density using a double beam spectrophotometer. **Conclusions:** The study concluded that the normal salivary glucose level was measured more accurately by the O-toluidine reagent method using a double beam spectrophotometer. Therefore, it can be concluded that the standard glucose solution needed for measuring normal glucose levels in the blood and saliva is in the ratio of 1:200–1:160.

Keywords: standard glucose solution, saliva, salivary glucose level, O-toluidine reagent, double beam spectrophotometer.

Background

Among the numerous systemic disorders, diabetes mellitus is most commonly encountered [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, 3]. Periodontal disease (gingivitis, periodontitis), dental caries, salivary dysfunction, dry mouth (xerostomia), oral mucosal diseases, and oral infections are all common complications in diabetics [4, 5]. Diabetes affects about 5% of all patients consulted in dental clinics, according to reports [6]. Saliva is important for maintaining the balance of the oral ecology. Saliva is referred to be the "mirror of the body"

since it functions as a health indicator not only in the mouth but also throughout the body [7]. In entire saliva, both locally produced and blood-derived indicators have been proven to be effective in the identification of a variety of systemic illnesses. Saliva analysis can be a cost-effective way to test large groups of people, and it may be a good option for patients who have trouble drawing blood or who have issues with compliance [8]. It bathes and mimics the position of bloodstream in the oral cavity because it includes serum constituents, which are assessed in routine blood tests to track health and diseases [9]. These components come from the salivary glands' local vasculature and gingival crevicular fluid [10]. It can be obtained non-invasively and by people with no experience, such as the patient. The fluid collection does not necessitate the use of any special equipment [11]. It's best for kids and patients who have fewer compliance issues, and it's a cost-effective way to test large groups of people [9]. The use of salivary biomarkers to diagnose various systemic diseases early reduces the need for more invasive care. It provides healthy oral health in a rejuvenated state by detecting oral health diseases early. Given that 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 on a regular basis should be considered.

Aims

To standardize the collection and measurement of glucose in saliva and its relationship with blood glucose concentration.

Materials and methods

Characteristics of participants

A study was conducted after obtaining ethical clearance from the institution. (CN-ABSM/EC/101/2021). Saliva was collected from the participants between 35 and 44 years and after calculation of the basal metabolic index (BMI) and considering the following inclusive and exclusive criteria.

Inclusive criteria

Healthy individuals without any past medical illness.

Individuals having BMI in range of 18.5–24.9.

Patients with age group between 35 and 44 years.

Exclusion criteria

Patients with medication which alters salivary flow and composition.

Patient under radiotherapy.

Pediatric patients.

Patient not willing to take part in the study.

Description of materials

BD Vacutainer fluoridated 2ml tube (Bectron drive, Franklin Lakes, NJ 07417 USA).

Centrifuge machine (Rohtek laboratory centrifuge-M8E).

O-toluidine reagent (119-93-7, *loba chemie*, India).

Glucose (S.L) standard solution (AGAPPE diagnostics, Ernakulam, Kerala, India).

Micro-pipette (dragon lab).

TCA (Emplura-UN1839).

Weighing balance (Sartorius scales).

PC-based double-beam spectrophotometer (SYSTRONICS-2202).

Principle

Proteins in saliva are precipitated by trichloroacetic acid (TCA), as it obstructs the estimation. The protein-free filtrate is produced by filtering the contents. It contains glucose of unknown concentration. In a boiling water bath, equal quantities of protein-free filtrate and

glucose solution are treated with an O-toluidine reagent (in acetic acid). The result is a blue-green N-glycosylamine derivative. The amount of glucose present determines the intensity of blue-green. Using a red filter (620 nm) on a PC-based double-beam spectrophotometer, the optical density values of all three solutions are read, and the quantity of glucose present in 100 ml of saliva is computed [12, 13].

Methodology

All salivary samples were taken between 9 and 11 a.m., 2 hours after breakfast, volunteers were asked not to have anything in between. Volunteers were asked to sit upright on the dental chair with the head kept forward, the patient was instructed not to swallow or speak. About 2 ml of a saliva were collected in sterile fluoridated container. The standard salivary glucose level was measured using the O-toluidine reagent method.

Preparation of protein: In a dry test tube, 3 ml of distilled water and 0.5 ml of saliva were combined thoroughly. A 1.5 ml of 10% TCA was added, mixed completely, and set alone for 10 minutes before being filtered into a dry test tube.

Development of color: Six test tubes were filled with standard glucose solutions ranging from 1 to 5 µg/ml, while a seventh test tube was filled with 1 ml of protein-free filtrate. A 5 ml of O-toluidine was added to each of these tubes and thoroughly mixed. The tubes were placed in a boiling water bath for 10 minutes, cooled, and the optical density was measured at 620 mm using a PC-based double-beam spectrometer [12, 13].

Results

After the analysis of the results obtained from the PC-based double-beam spectrophotometer using the O-toluidine reagent method, it was confirmed that the amount of glucose standard solution needed to measure the standard glucose level is in the range of 1–5 µg/ml with the OD reading ranging from 0.5 to 0.589 with the blank OD reading of 0.489 at 620 nm and the absorbance value ranging from 0.011 to 0.1 (Table 1).

When the results as shown in Table 1 were plotted on the graph with optical density reading obtained from a PC-based double-beam spectrophotometer along the y-axis and the concentration of standard glucose solution (AGAPPE) along the x-axis shows a linear increase as the glucose concentration is increased with R^2=0.9584 (Figure 1).

The salivary samples collected from the individuals who participated in the study who did not have a significant medical history and were in the age group of 35–44 years as per inclusive and exclusive criteria were collected in a sterile fluoridated tube and the salivary glucose level was measured using O-toluidine reagent method and readings was tabulated as shown in Table 2 as obtained from PC-based double-beam

Table 1: Optical Density and Absorbance of Glucose Standard solution using PC-based double-beam spectrophotometer.

S. No.	Volume of 30 ml of 10% TCA diluted to 100 ml (ml)	Volume of glucose	Volume of distilled water	Concentration of glucose (µg/ml)	Volume of O-toluidine reagent (ml)	Keep in boiling water (minutes)	Optical density at 620 nm	Absorbance (test OD-sample OD)
Blank	1	-	-	-	5	10	0.489	
1	-	10	990	1	5	10	0.500	0.011
2	-	20	980	2	5	10	0.521	0.032
3	-	30	970	3	5	10	0.558	0.069
4	-	40	960	4	5	10	0.561	0.072
5	-	50	950	5	5	10	0.589	0.1

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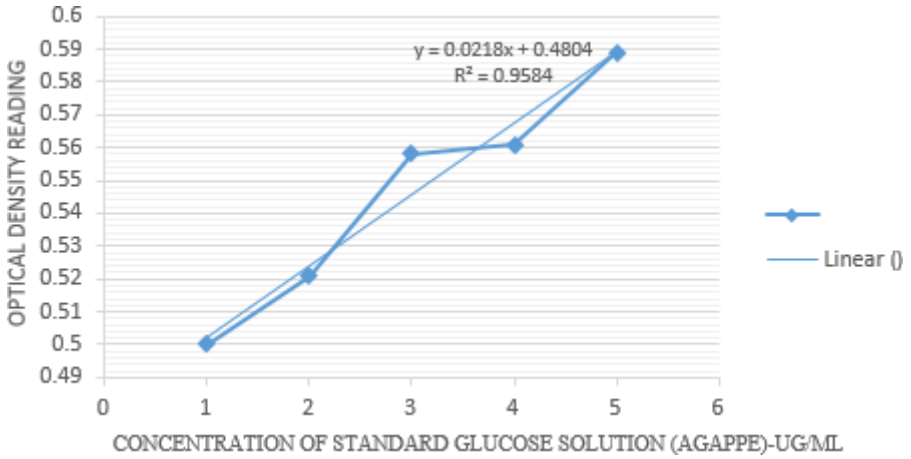

Figure 1: R value obtained by plotting Concentration of Standard glucose solution against the Optical Density reading obtained using PC-based double-beam spectrophotometer.

Table 2: Optical Density and Absorbance of samples using PC-based double-beam spectrophotometer.

S. No.	Volume of protein free filtrate sample (ml)	Volume of O-toluidine reagent (ml)	Keep in boiling water bath (minutes)	Absorbance OD at 620 nm	Absorbance (test OD-sample OD)
1	1	5	10	0.584	0.095
2	1	5	10	0.558	0.069
3	1	5	10	0.564	0.075
4	1	5	10	0.566	0.077
5	1	5	10	0.554	0.065
6	1	5	10	0.568	0.079
7	1	5	10	0.550	0.061
8	1	5	10	0.562	0.073
9	1	5	10	0.564	0.075

spectrophotometer. Triplication was done for each sample and the results obtained (Table 2) was within the normal salivary glucose level as obtained in Table 1.

With all the findings obtained and evaluated the results, it shows that standard salivary glucose level can be measured accurately by the O-toluidine reagent method using a PC-based double-beam spectrophotometer at 620 nm. The normal blood glucose level after 90–120 minutes of fasting is 100–160 mg/100 ml and that of saliva is 0.5–1 mg/100 ml by converting the value obtained [14]. From the results obtained and from the standard levels the ratio of blood glucose level and salivary glucose level was found to be 1:200–1:160.

Discussion

Saliva is becoming more and more effective as a diagnostic aid. It's a body fluid with a unique structure and function [15]. Human saliva is a specific secretion produced by the major and minor salivary glands that aid in the regular physiologic activities of oro biological tissues. The value of precision in saliva measurements was stressed by Dawes et al. 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 the hours of 9:00 and 11:00 a.m. [16]. The examination of biochemical contents in saliva aids in the diagnosis of disorders

of the oral cavity as well as the monitoring of overall health.

There is a lot of evidence to support the use of saliva in the management of hyperglycemia. Glucose is a small molecule that spreads easily across semi-permeable diaphragms. Changes in the basement membrane of blood vessels in diabetic patients cause saliva to increase the distribution of blood glucose [17]. In another study, Borg A. 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 people [18]. 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 [19]. 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 [20].

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 [21]. Ivanovski et al., conducted a study among xerostomic diabetic patients and concluded that even if the patient had xerostomia, which is a complication of diabetes the association between blood and salivary glucose levels was statistically important [22].

Salivary and blood glucose levels are linked and can be useful in diabetes management. Despite the intrusive nature of today's glucose control procedures, saliva can be used as a non-invasive diagnostic fluid to help solve this issue. As a result, saliva testing overcomes all of the disadvantages of venepuncture and allows for the testing of people of all ages [23]. 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 to blood and urine tests [24].

Conclusion

The study concluded that the normal salivary glucose level was measured more accurately by using the O-toluidine reagent method using a PC-based double-beam spectrophotometer using a fluoridated tubes for the collection of saliva. The standard glucose concentration (AGAPPE) required to measure the standard glucose level of saliva is 1–5 µg/ml. Therefore, it can be concluded that the standard glucose solution needed for measuring normal glucose levels in the blood and saliva is in the ratio of 1:200–1:160.

List of abbreviations

TCA – Trichloro acetic acid
OD – Optical density
CN – Certificate number
BMI – Body mass index
R – Read output in excel sheeth

Conflict of Interest

The authors declare no conflict of interest.

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