Innovations

Evaluating the Correlation between Blood and Salivary Glucose Tolerance Test Levels in Young Adults: A Comparative In-vitro Study

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Abstract

Objectives: *This study aims to estimate and compare the levels of serum and salivary glucose tolerance test (GTT) among young adults.* ***Materials and Methods:*** *The study was conducted in the Department of Oral Pathology and Microbiology after obtaining approval from the institutional ethical committee. A total of 40 samples were studied, comprising two groups: 20 males and 20 females. Age and gender-matched healthy individuals were included. Serum and un-stimulated salivary GTT levels were analyzed using the glucose oxidase-peroxidase (GOD-POD) enzymatic method. Samples were collected during fasting and at 1-hour intervals for 2 hours. The samples were subjected to centrifugation at 10,000 rpm, and the supernatant was separated and used for analysis. The final values were spectrophotometrically analyzed at 540 nm.* ***Results:*** *Data obtained were entered into an Excel sheet and subjected to statistical analysis using the one-way ANOVA test. A highly significant P-value ($P < 0.05$) was observed in females compared to males. A distinct difference was observed between blood and salivary glucose levels.* ***Conclusion:*** *This study attempted to compare salivary and serum glucose levels in young adults, revealing that salivary glucose levels can potentially serve as a diagnostic aid in analyzing glucose levels.*

Keywords: *Diabetes mellitus, glucose oxidase-peroxidase, non-invasive, salivary glucose level, serum glucose level*

Introduction:

Diabetes mellitus (DM) is a chronic condition resulting from a disruption in carbohydrate metabolism, requiring lifelong management. The complexities of diabetes often make it difficult to cure. Poorly managed diabetes can lead to dysfunctions and imbalances in tissue and organ metabolism, resulting in weakened immunity and overall health, along with the onset of complications. These complications can cause significant discomfort and pose potentially life-threatening risks to affected individuals (1).

Early diagnosis of diabetes allows for effective management through simple interventions like dietary adjustments.(2) However, if left undetected until advanced stages, diabetes can lead to severe conditions such as heart disease, kidney disease, vision impairment, and paralysis. It's important to note that diabetes typically begins affecting the body 4–7 years before it is clinically diagnosed(3).

Saliva serves as a valuable biofluid in clinical settings, offering insights for innovative methods in diagnosing, predicting, monitoring, and treating oral and systemic illnesses. Its non-invasive collection method makes it particularly advantageous. The Salivary Glucose Tolerance Test (GTT) has the potential to replace venous blood draws in specific medical situations and facilitates the early detection of Diabetes mellitus(1).

In clinical practice, it's advisable to opt for an oral glucose tolerance test (OGTT) for diagnostic confirmation only when casual blood glucose readings fall within an uncertain range, meaning they are neither definitive for nor against a diabetes diagnosis, and when fasting blood glucose levels are below the threshold for diagnosing diabetes.

Conducting an OGTT involves measuring blood glucose levels before fasting and two hours after ingesting a 75g oral glucose solution. Utilizing saliva as a biological sample offers the benefits of rapid, straightforward, and non-invasive collection. Furthermore, oral fluid sampling ensures safety for both the operator and the patient, with convenient and cost-effective storage options. This method aids in diagnosing diseases and evaluating the severity of certain illnesses(4).

The present study aims to assess and compare the levels of GTT in both serum and saliva within the young adults. The study's objectives include evaluating salivary GTT levels, estimating serum GTT levels, and comparing the GTT levels in serum and saliva among young adults in this demographic.

Materials and Methods:

Forty apparently healthy individuals (20 men and 20 women) were asked to participate in an interventional study, conducted at the Department of Oral and Maxillofacial Pathology, Adhiparasakthi Dental College and Hospital. Age and

gender matched healthy individuals aged 18 to 20 years were included in this study, where as individuals with systemic illness, those undergoing antidepressive & antihistamine therapy, individuals undergoing chemotherapy & radiotherapy, smokers and alcoholics were excluded in this study(5).

After consuming an aqueous solution (300 ml) containing 75 g of glucose within 5 minutes of the test, the participants thoroughly washed their mouths with tap water and brushed their teeth. Venous blood and saliva were concurrently drawn from each participant prior to, during, and two hours following their consumption of glucose(6).

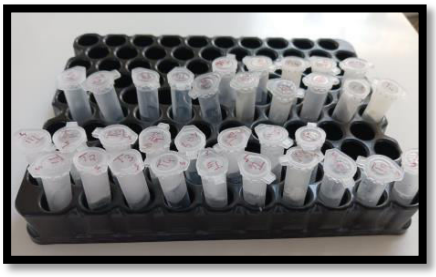
Between 8:00 and 11:00 AM, samples were gathered in a quiet room while they were at rest. Without chewing motions, expectoration was used to collect unstimulated salivary samples into a plastic tube. Venipuncture was used to draw blood specimens, which were then collected and allowed to coagulate in 10-ml glass vacuum tubes without any additives.

Following a centrifugation of the blood and saliva at 10,000 rpm, the serum and saliva supernatants were separated. Saliva and serum were collected, and then their glucose levels were measured using spectrophotometry at 540 nm(7).

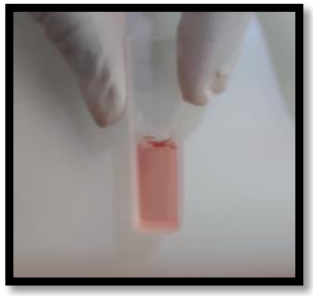
Glucose is oxidized to gluconic acid while oxygen is simultaneously reduced to hydrogen peroxide by the enzyme glucose oxidase. Hydrogen peroxide is then split to form water and nascent oxygen by the enzyme peroxidase. That nascent oxygen reacts with 4-aminoantipyrine, and in the presence of phenol, this reaction produces quinoneimine, which is a colored compound that can be analyzed. GOD is the catalyst that converts glucose into hydrogen peroxide and gluconic acid. Phenol + 4 aminoantipyrine + hydrogen peroxide = Quinoneimine Quinoneimine creates a red colour complex that a colorimeter reads to determine the value(8).

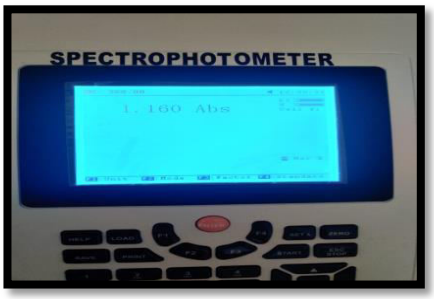
Methodology

Saliva samples **Centrifuged saliva sample** **Centrifuged blood sample**



Spectrophotometer analysis





ARMAMENTARIUM

Sterile sample Container

Eppendorf tube

Centrifuge

GOD-POD reagent Kit

Glucose

Spectrophotometer

Results:

Thirteen women and seventeen males made up the study sample, with a mean age of 18 to 20 years.All the salivary samples were analyzed using spectrophotometry. The mean serum GTT levels were highest in 1 hr after glucose intake. The mean salivary GTT levels were highest in 1 hr after glucose intake(Table: 1).

Table: 1 Average mean values of salivary and serum fasting, 1hr & 2hr samples

Salivary fasting	Salivary P1	Salivary P2	Serum fasting	Serum P1	Serum P2
47.51	58.23	44.1	88.65	96.95	86.27

On comparing within the groups (Table:2), using paired samples test, the mean serum GTT levels in fasting, 1 hr and 2 hr samples were significantly elevated (p value<0.05) than mean salivary GTT levels in both group 1 and group 2. On intergroup comparison using ANOVA test, a significant p value (<0.05) was derived.

Graph : 1 Serum and Salivary GTT levels among young adults

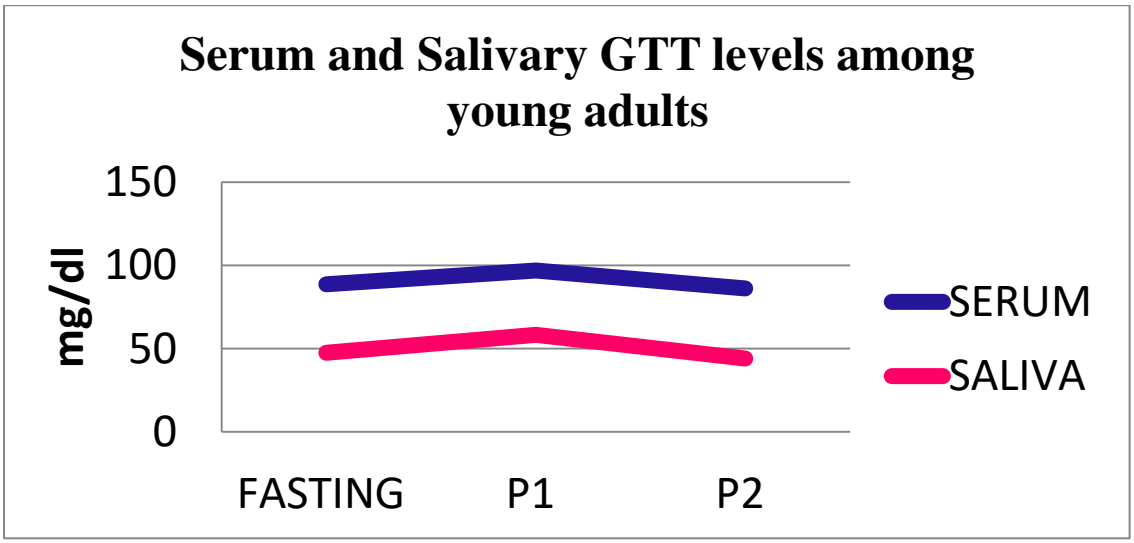


Table: 2 Comparison of salivary glucose levels and serum glucose levels among both groups

GROUP	PARAMETER	MEAN DIFFERNCE	F	95% CONFIDENCE INTERVAL		SIG
				LOWER	UPPER	
FASTING GLUCOSE	SALIVA	-.09085	1.327	-0.27	.08908	.257

	SERUM	5.50000	.002	1.15	9.85003	.965
P1 GLUCOSE	**SALIVA**	.07290	3.393	-0.04608	.19188	**.043**
	SERUM	13.00000	4.645	9.27602	16.72398	**.038**
P2 GLUCOSE	**SALIVA**	-.08315	1.694	-.23832	.07202	**.053**
	SERUM	4.15000	.807	-.76477	9.06477	**.055**

Discussion:

Diabetes mellitus is a prevalent worldwide condition, and while numerous diagnostic tools exist, the blood glucose test is considered the benchmark for diagnosis. Nonetheless, blood collection, which is necessary for this method, is invasive(9).

Saliva serves as a valuable indicator for the early detection of diseases, leading to more efficient diagnosis and treatment or monitoring of systemic illnesses like diabetes mellitus(10). Additionally, the composition of saliva may reflect the overall health condition of the patient rather than just their oral health. The connection between serum glucose levels and salivary glucose levels was first observed by Kortuem(11) in 1944, followed by Shannon et al.(12) in 1960, Englander et al.(13) in 1963, and Campbell(14) in 1965.

There is glucose in saliva and can be detected in several experiments(15).(Birkhed D, Berntorp K, Lindgärde F, Matsson L.) Glucose, being a small molecule, can readily pass through semipermeable membranes, leading to elevated levels of glucose in saliva.(4) This can consequently lead to changes in salivary glucose levels and an increased vulnerability to oral diseases. Unstimulated whole saliva has been predominantly utilized in diagnostic research due to its natural dilution and avoidance of pH alterations seen in stimulated saliva.(8)

Basement membrane permeability facilitates passage of small molecules like glucose from serum into saliva via gingival crevices(16). Hence saliva samples are

recently being studied and considered as a novel diagnostic tool in detection of glucose levels. The present study has been attempted to detect the salivary GTT levels among young adults.

Mirzaii-Dizgah MH et al,(17) discovered that during the glucose tolerance test, the healthy participants' salivary glucose levels rose in 60 minutes and fell in two hours which is similar to our study which also showed elevation of salivary glucose levels after 1 hr which returned to normal after 2 hrs similar to serum GTT results in healthy individuals.

Our study seems to be one among the few studies conducted to estimate the salivary GTT levels among young healthy adults and further studies can be conducted using larger sample size for confirmation and standardization.

Conclusion:

The present study underscores the potential of saliva as a non-invasive diagnostic tool for monitoring glucose levels in young, healthy adults. Our findings align with previous research, demonstrating that salivary glucose levels rise and fall in a pattern similar to blood glucose levels during a glucose tolerance test. This correlation suggests that saliva can effectively reflect systemic glucose changes, offering a less invasive alternative to traditional blood tests. The utilization of unstimulated whole saliva further enhances the reliability of this method, avoiding the complications associated with stimulated saliva samples.

Given the promising results, further research with larger sample sizes is warranted to confirm and standardize the use of salivary glucose tests. By establishing robust protocols and validation, saliva could become a practical, accessible, and patient-friendly option for diabetes diagnosis and management. This advancement could lead to more efficient early detection, improved monitoring, and better overall management of diabetes mellitus. In summary, unstimulated saliva can be used in place of serum in the GTT for the diagnosis of diabetes mellitus.

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Conflicts of interest: No conflicts of interest exist.

References:

1. *Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: a new laboratory tool for diagnostic and basic investigation. Clin Chim Acta. 2007 Aug;383(1–2):30–40.*
2. *Naik R, Mujib B.R A, Raaju UR, Telagi N. Assessing Oral Candidal Carriage with Mixed Salivary Glucose Levels as Non-Invasive Diagnostic Tool in Type-2 Diabetics of Davangere, Karnataka, India. J Clin Diagn Res. 2014 Jul [2024 Mar 30];8(7):ZC69–72.*
3. *Ping W, Yi T, Haibao X, Farong S. A novel method for diabetes diagnosis based on electronic nose1. Biosensors and Bioelectronics. 1997 Nov 1 [2024 Mar 26];12(9):1031–6.*
4. *Mirzaii-Dizgah MH, Mirzaii-Dizgah I, Mirzaii-Dizgah MR. Oral glucose tolerance test in unstimulated saliva of healthy individuals. European Journal of General Dentistry. 2016 Jan [2024 Mar 30];5(01):15–8.*
5. *Gupta S, Sandhu SV, Bansal H, Sharma D. Comparison of Salivary and Serum Glucose Levels in Diabetic Patients. J Diabetes Sci Technol. 2014 Oct 7 [2024 Mar 30];9(1):91–6.*
6. *Soares MS, Batista-Filho MM, Pimentel MJ, Passos IA, Chimenos-Kustner E. Determination of salivary glucose in healthy adults. Med Oral. 2009 [2024 Apr 2];e510–3.*
7. *Ragunathan H, Aswath N, Sarumathi T. Salivary Glucose Estimation: A Noninvasive Method. Indian Journal of Dental Sciences. 2019 Mar [2024 Mar 30];11(1):25.*
8. *López ME, Colloca ME, Páez RG, Schallmach JN, Koss MA, Chervonagura A. Salivary characteristics of diabetic children. Braz Dent J. 2003;14(1):26–31.*
9. *Rp A. Noninvasive Method for Glucose Level Estimation by Saliva. J Diabetes Metab. 2013 [2024 Mar 30];04(05).*
10. *Kumar S, Padmashree S, Jayalekshmi R. Correlation of salivary glucose, blood glucose and oral candidal carriage in the saliva of type 2 diabetics: A case-control study. Contemp Clin Dent. 2014 [2024 Mar 30];5(3):312–7.*
11. *Shannon IL, Prigmore JR, Brooks RA. Glucose concentrations in parotid fluid and blood serum following intravenous glucose loading. Oral Surgery, Oral Medicine, Oral Pathology. 1960 Aug [2024 Mar 30];13(8):1010–2.*
12. *Englander HR, Jeffay AI, Fuller JB, Chauncey HH. Glucose Concentrations in Blood Plasma and Parotid Saliva of Individuals with and without Diabetes Mellitus. J Dent Res. 1963 Sep [2024 Mar 30];42(5):1246–1246.*
13. *Campbell MJA. Glucose in the saliva of the non-diabetic and the diabetic patient. Archives of Oral Biology. 1965 Mar [2024 Mar 30];10(2):197–205.*
14. *Andersson AB, Birkhed D, Berntorp K, Lindgärde F, Matsson L. Glucose concentration in parotid saliva after glucose/food intake in individuals with*

glucose intolerance and diabetes mellitus. European J Oral Sciences. 1998 Oct [2024 Mar 30];106(5):931–7.

15. *Barros SP, Williams R, Offenbacher S, Morelli T. Gingival Crevicular as a Source of Biomarkers for Periodontitis. Periodontol 2000. 2016 Feb [2024 Apr 2];70(1):53–64.*
16. *Mirzaii-Dizgah MH, Mirzaii-Dizgah MR, Mirzaii-Dizgah I. Serum and Saliva Myelin Basic Protein as Multiple Sclerosis Biomarker. Basic Clin Neurosci. 2021 [2024 Apr 2];12(3):309–14.*