Innovations

Comparative Estimation of Serum and Salivary GTT Levels among Type II Diabetes Mellitus Individuals- An Invitro Study

[*1]Dr.A.Beeula,[2]Dr. Sastimani.P.M, [3]Dr. S. Shamala, [4]Dr. Adhithya.B, [5]Dr. Kokila.S

[1,4,5]Senior lecturer, [2]Post graduate, [3]Professor and Head
[1,2,3,4&5]Department of Oral Pathology and Microbiology, Adhiparasakthi dental college and hospital, Melmaruvathur, Tamilnadu, India

Corresponding Author: **Dr.A.Beeula**

Abstract

Background: *Diabetes mellitus, a metabolic disorder linked to heredity withabnormally elevated blood glucose levels has been reported with highest incidence rate in India being described as the capital hub globally. Though serum GTT(Glucose tolerance test) level estimations are considered gold standard in diagnosis of diabetes mellitus reports have suggested that salivary glucose levels can also be considered in estimation of glucose levels as it seems to be a non -invasive procedure.* ***Aim:*** *To estimate and compare salivary and serum GTT levels among Type 2 Diabetes Mellitus individuals.* ***Materials and Methods:*** *The study was conducted in the department of Oral Pathology and microbiology after the approval from institutional review board. Total of 80 samples comprising of 2 groups: 40 controls (Group 1) and 40 type 2 Diabetes mellitus (Group 2) age and gender matched individuals with known diabetes mellitus were included in the study. Blood and unstimulatedsalivary GTT levels were analyzed using GOD-POD (Glucose oxidaseand Peroxidase)enzymatic method. The samples were collected during fasting; 1-hour interval and 2 hours interval (post prandial). The samples were centrifuged at 10,000 rpm and we respectrophotometrically analyzed at 540nm.* ***Results:*** *The one-way ANOVA test was used for statistical analysis. On comparison between the groups, postprandial salivary and serum samples of type 2 diabetes mellitus showed highest mean value (**255.95 mg/dl**) and (**90.34 mg/dl**) than other groups and with a statistically significant P value of < 0.05.* ***Conclusion:*** *As saliva analysis is a non- invasive diagnostic technique, it can be used for monitoring glycemic status of type 2 diabetes mellitus status and can be used as an alternative to serum samples. In this study, an attempt has been*

made to estimate and correlate the salivary GTT levels to compare between diabetics and non-diabetics and also to obtain a salivary glucose standard value.

Key words: *Saliva, Glucose Tolerance Test, Diabetic mellitus, diagnostic tool*

Introduction:

Saliva is a vital, multifunctional biological fluid secreted by major and minor salivary glands(1), comprising of 90% water, 10% enzymes, electrolytes, antimicrobial agents, proteins, hormones etc. Recently saliva is used widely among researchers due to a non-invasive approach of collection technique (6). Serumis a gold standard diagnostic tool in monitoring disease states as it plays a vital role in various physiological and diagnostic functions (4) as unlike plasma, serum does not contain fibrinogen and other clotting factors, making it ideal for many laboratory tests.(3)Diabetes mellitus is a chronic metabolic disorder characterized by high levels of glucose in the blood due to the body's inability to produce sufficient insulin (2). It is reported that globally 439 million people (7.7% of the population) are expected to have diabetes by 2030. By 2025, there will be 70 million diabetics in India increasing from the current 41 million of the total population (3). Type 2 diabetes mellitus is most common resulting from insulin resistance or insulin deficiency.(8)Glucose tolerance test is a diagnostic tool commonly used to diagnose diabetes and pre- diabetes.(9) Recent studies have reported that saliva can be used in diagnosing diabetes mellitus due to its easy and non- invasive mode of collection when compared to serum.(10)Hence, this current study was attempted to estimate and compare the salivary and serum GTT levels among type 2 diabetes mellitus individuals in Indian population.

Methodology:

The study was approved from Institutional Review Board(2023/IRB-OP-01/APDCH). An informed consent was obtained from every individual before sample collection and the patient was verbally informed regarding the procedure.A total of 80 saliva and serum samples wasobtained and weregrouped intoGroup 1, normal healthy individuals (n=40) and group 2, type II Diabetes mellitus individuals (n=40). Demographic data was collected with age and gender matched individuals of age between 30 to 60 years with known type 2 diabetes mellitus were included.Subjects under medication for diabetes mellitusor any other systemic illness were excluded from the study.

Study setting: The samples were collected from out -patient department of APDCH and were stored at -20 degree Celsius. Spectrophotometric analysis of the samples was done in Department of oral pathology APDCH.

Sample collection: A 300 ml aqueous solution containing 75g of glucose was used for the GTT, and it was consumed in 5 minutes interval.Salivary and blood sample was collected at fasting, 1 hr interval followed by 2 hr interval.An informed consent is obtained from the patients before collecting samples.(7)The blood samples were also collected at fasting and at an interval of 1 hr and 2hrs(5). The collected samples were centrifuged and serum was separated and stored at -20 degree Celsius.The saliva and blood samples collected were subjected to centrifugation at 8000 rpm for 12mins and the supernatants collected were stored at -20°c(8). Salivary and blood GTT levels were analyzed using GOD-POD method. The final readings were analyzed using spectrophotometer at 560nm wavelength.(fig 1, 2, 3, 4)

Procedure: Serum and Salivary GTT levels were analyzed using GOD-POD method. The reagents are pre-warmed to reaction temperature. 1 ml of glucose reagents is added to the blank (B), standard (S) and test sample (T) marked centrifuge tubes. Later,provided standard of 0.010 was added to the standard centrifuge tube and mixed well. Test samples are added to the marked centrifuge tubes and mixed well and incubate for 10min at 37 degree Celsius or 20 to 25 min at 15 -25 degree Celsius. After incubation, the values are read using spectrophotometer at 560nm wavelength.

Results

Statistical Analysis: All thedata's were entered in the excel sheet, Descriptive analysis was done. Inter-grouping comparison was analyzed using one-wayANOVA test. Multiple comparison between the groups were analyzed using ANOVA. All the final data was done using SPSS 21.00.

The mean GTT levels were higher in serum (255.95) when compared to saliva(90.34) in both diabetic and non-diabetic subjects. The mean serum GTT levels were highest in 2hr after glucose sample (255.95) followed by 1 hr (209.15) and fasting sample (180.42**).** The mean salivary GTT levels were highest in 2hr (90.34) after glucose sample followed by 1 hr(85.17) and fasting sample (73.19). (Table and graph 1)

Discussion

Diabetes mellitus is considered as a universally wide-spread metabolic disease. Blood glucose estimation is the most popular technique for analyzing glucose levels and there are ranges of diagnostic devices available in the market to determineglucose levels.As with advanced technologies in medical field there is still demand in establishing a noninvasive procedure to analyze glucose levels. Standardizing salivary glucose in diagnosing diabetes mellitus providesa lightening change globally and unstimulated saliva is being accepted as a diagnostic tool because of its less probability of dilution and modulation in PH.

The process of determining whether a patient can consume and store glucose normally is called a glucose tolerance test (GTT). The test is typically used to screen for uncommon abnormalities of glucose metabolism, decreased pancreatic beta cell activity and diabetes mellitus. (19) An oral glucose tolerance test (OGTT), which is the most widely used variant of the test, involves taking a standard dosage of glucose orally (75 grams) and checking blood levels at intervals of 1hr and 2 hours later. Over the years, numerous versions of the GTT have been developed for a variety of uses.
Glucose molecules are readily detected in secretory saliva in conditions of hyperglycemia as they tend to diffuse across the altered basement membrane of blood vessels and enter saliva according to Harrison and Bowen's.(18)It has been established that hyperglycemia leads to increased glycosylation end products, known as "Advanced Glycosylation end products (AGEs)." They form a cross-linkage between proteins such as collagen and extracellular matrix proteins, leading to basement membrane modification and hence endothelial dysfunction. This alteration makes the microvasculature structure more permeable allowing diffusion of glucose into saliva.

Although limitations still exist in regarding the replacement of plasma with saliva in diagnosing diabetes mellitus because of lower salivary glucose concentrations various studies have been attempted in the linkage between serum and saliva in diagnosing diabetes. In this study we have compared the serum and salivary glucose levels in recommended intervals as in glucose tolerance test (GTT) among type 2 diabetes individuals.Our results revealed major variations of high levels of salivary glucose concentrations in type 2 DM individuals. This was similar to the study by Jurysta et al in 2009 where he re-evaluatedthe salivary glucose concentration and excretions in unstimulated and mechanically stimulated saliva in both normal and diabetic subjectsincluding males and females and reported higher levels of glucose concentration among male diabetes mellitus subjects(13). Abhikshyeet P in 2012compared saliva samples with blood glucose and glycated hemoglobin (HbA1c) in diabetic subjects and found the positive variation between salivary and serum glucose levels in diabetic individuals. (15)Naik et al in 2015 and Darwazeh et al in 2014 correlated serum and salivary glucose levels with regards to duration, age and gender and the results showed appreciable variations with all the parameters analysed. (16)

Few studies showed insignificant differences between type-1 and type-2 diabetes mellitus.It can be due to higher glucose concentrations and less permeability associated with membranopathy. (17) Nevertheless, further studies with a larger sample size, gender and age grouping and also including type 1 and 2 diabetes samples are needed to evaluate the diagnostic value of salivary glucose levels for early diagnosis and better treatment planning as saliva stands to be a non -invasive tool.The presence of glucose in the saliva of the diabetics probably reflects the high serum

glucose concentrations. An earlier study indicated that the salivary glucose concentration was lower during the period of better metabolic control (Reutervinget al., 1987). Therefore, in well-controlled individuals with altered glucose metabolism, salivary gland function is not significantly impaired. The presence of glucose in the saliva of the diabetics probably reflects the high serum glucose concentrations. An earlier study indicated that the salivary glucose concentration was lower during the period of better metabolic control (Reutervinget al., 1987). Therefore, in well-controlled individuals with altered glucose metabolism, salivary gland function is not significantly impaired. The presence of glucose in the saliva of the diabetics probably reflects the high serum glucose concentrations. An earlier study indicated that the salivary glucose concentration was lower during the period of better metabolic control (Reutervinget al., 1987). Therefore, in well-controlled individuals with altered glucose metabolism, salivary gland function is not significant.

Conclusion

A better understanding of metabolic homeostasis in healthy individuals and the altered metabolic phenotype in Type 2 diabetes will likely lead to the development of better treatments for Type 2 diabetes(20).The present study showed elevated glucose levels in GTT in both serum and saliva of both diabetic and healthy subjects. Hence Salivary GTT level could be considered as a potentially non-invasive diagnostic tool in monitoring glycemic status in diabetic individuals. Human saliva contains α amylase and lipase, substances that may play a role in starch digestion and decomposition and triglyceride breakdown in neonates born with pancreatic dysfunction. Salivary mucins play a significant role in lubricating the intraoral structures and help forming a barrier against microbial invasion(5). This study is one among the few attempted to evaluate and compare the salivary and serum GTT levels in diabetic subjects. The results showed significant elevated values of salivary glucose similar to serum glucose levels and further studies may be performed with larger sample size for further evaluation and standardization.

Conflict of interest: Nil
Funding: Nil

Reference:

1. *Chen L, Magliano DJ, Zimmet PZ. The worldwide epidemiology of type 2 diabetes mellitus-present and future perspectives. Nat Rev Endocrinol. 2012;8(4):228-236*
2. *Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes. 2015 Jun 25;6(6):850-67.*
3. *Banday MZ, Sameer AS, Nissar S. Pathophysiology of diabetes: An overview. Avicenna J Med. 2020 Oct 13;10(4):174-188.*

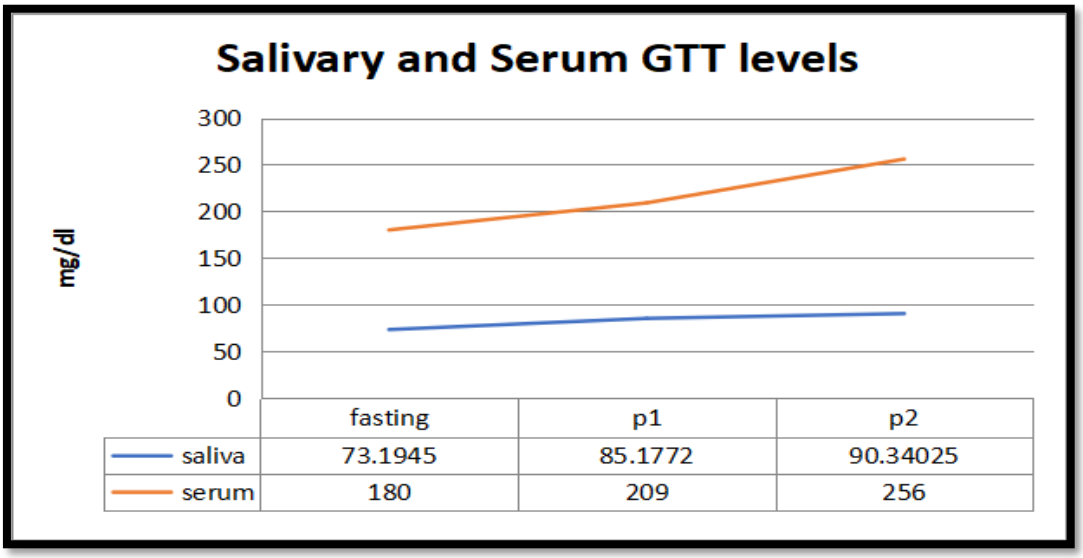
4. *Ramachandran A, Snehalatha C. Current scenario of diabetes in India. J Diabetes. 2009;1(1):18-28.*
5. *Martina E, Campanati A, Diotallevi F, Offidani A. Saliva and Oral Diseases. J Clin Med. 2020 Feb 8;9(2):466.*
6. *Vila T, Rizk AM, Sultan AS, Jabra-Rizk MA. The power of saliva: Antimicrobial and beyond. PLoSPathog. 2019 Nov 14;15(11):e1008058.*
7. *Radicevic AB, Dozic R, Todorovic T, Dozic I. Biochemical markers in saliva of patients with diabetes mellitus. Serbian Dental Journal. 2012; 59(4):198-204.*
8. *Eyth E, Basit H, Swift CJ. Glucose Tolerance Test. 2023 Apr 23. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 30422510.*
9. *Jagannathan R, Neves JS, Dorcely B, Chung ST, Tamura K, Rhee M, Bergman M. The Oral Glucose Tolerance Test: 100 Years Later. Diabetes Metab SyndrObes. 2020 Oct 19;13:3787-3805.*
10. *Ben-Aryeh H, Cohen M, Kanter Y, Szargel R, Laufer D. Salivary composition in diabetic patients. J Diabet Complications. 1988; 2(2):96-99. 20.*
11. *Naik VV, Satpathy Y, Pilli GS, Mishra MN. Comparison and correlation of glucose levels in serum and saliva of patients with diabetes mellitus. Indian J Pub Health Res Dev. 2011;2(1):103-10*
12. *Jurysta C, Bulur N, Oguzhan B, et al. Salivary glucose concentration and excretion in normal and diabetic subjects. J Biomed Biotechnol. 2009;2009:1-6*
13. *Sharon A, Ben-Aryeh H, Itzhak B, Yoram K, Szargel R, Gutman D. Salivary composition in diabetic patients. J Oral Med. 1985;40(1):23-26.*
14. *Abhikshyeet P, Ramesh V, Oza N. Glucose estimation in the salivary secretion of diabetes mellitus patients. Diabetes MetabSyndrObes. 2012;5:149-154*
15. *Darwazeh AM, MacFarlane TW, McCuish A, Lamey PJ. Mixed salivary glucose levels and candidal carriage in patients with diabetes mellitus. J Oral Pathol Med. 1991;20(6):280-283*
16. *Forbat LN, Collins RE, Maskell GK, Sönksen PH. Glucose concentrations in parotid fluid and venous blood of patients attending a diabetic clinic.* Journal of the Royal Society of Medicine.
17. *Gupta V, Kaur A. Salivary glucose levels in diabetes mellitus patients: A case–control study. Journal of Oral and Maxillofacial pathology. 2020 Jan 1;24(1):187.*

Table & graph legends:

Table 1:inter grouping difference with P value

Graph 1: Serum and salivary GTT levels

Figure legends:
Figure1: Serum and saliva sample
Figure 2: Centrifuged sample
Figure 3: color change after adding reagent to the salivary sample
Figure 4: spectrometer analysis



Graph 1: Salivary and Serum GTT Levels

GROUP	**Salivary fasting (MEAN)**	**Serum fasting** (mean)	**Salivary p1(mean)**	**Serum p1** (mean)	**Salivary p2** (mean)	**Serum p2** (mean)
Control N=40	64.22	79.4	69.29	86.35	68.38	98.15
Type 2-DM N=40	**73.19**	**180.42**	**85.17**	**209.15**	**90.34**	**255.95**

Table 1: salivary and serum inter grouping differences with p value

FIG 1: Blood and Whole Saliva Sample

Fig 2: Centrifuged Saliva Sample

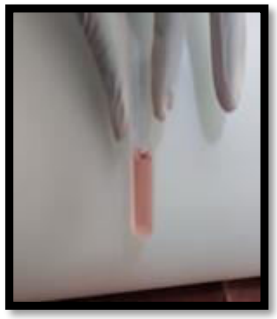


Fig3:color changeafter adding Reagent

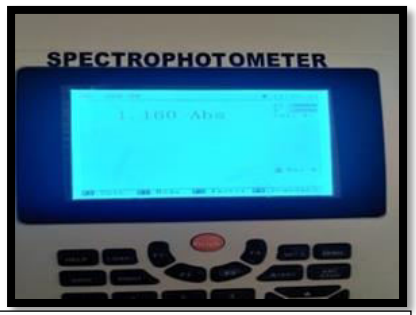


Fig 4: Spectrophotometric analysis