Effect of Time Interval between Collection, Plasma Separation and Analysis and the Effect of Temperature on the Laboratory Results of Plasma Glucose Estimation

Ashish Agarwal[1*], Poornima Ajay Manjrekar[2], Afzal Ahmad[1] and Charu Yadav[3]

[1]*Department of Biochemistry, GS Medical College and Hospital, Pilkhuwa, U.P., India.*
[2]*Department of Biochemistry, Kasturba Medical College, Mangalore, MAHE, Manipal, Karnataka, India.*
[3]*Department of Biochemistry, Lady Hardinge Medical College, New Delhi, India.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors Ashish Agarwal and PAM designed and drafted the article. Author Afzal Ahmad did the data acquisition, analysis and interpretation. Author CY critically revised the article. Author Afzal Ahmad managed accountability. All authors finally approved manuscript version to be published.

Original Research Article

ABSTRACT

Introduction: In a clinical laboratory, consistency of test results cannot be solely achieved by controlling the accuracy in the analytical phase of glucose estimation alone. Considering the high chances of pre-analytical errors, due consideration has to be given for storage criteria as well.
Aims: Aim is to find out the quantitative alterations and the useful time interval between collection and analysis and the effect of temperature on the laboratory results of plasma glucose.
Study Design: Cross sectional study.
Place and Duration of Study: Study was conducted at Department of Biochemistry, Kasturba Medical College Hospitals, Mangaluru between 2015 and 2016.
Methodology: In this study, 17 apparently healthy volunteers aged 20-30 years were enrolled with their consent. Plasma glucose levels were estimated by glucose oxidase- peroxidase kit method in

Corresponding author: E-mail: shivashish3001@gmail.com;

semi-autoanalyzer at different temperatures and at different time intervals after collection and at room temperature but separating plasma at different time intervals and correlating it with the initial value.

Results: The mean of plasma glucose when estimated immediately after separation of plasma was 78.16 ± 13.28 mg/dL. Only when glucose was estimated within 2 hrs when stored at 2-8° Celsius after plasma separation (71.57 ± 12.64), non- significant difference was noted (p value – 0.696) otherwise as the temperature and time interval for estimation increases, the value of glucose decreases significantly. The effect of delayed separation of plasma showed that as the time of separation increases the value of glucose decreases significantly in the sample.

Conclusion: Most mistakes occur before the samples are analysed, either during sampling or preparation for analysis. Thus, proper storage temperature and time must be considered for plasma glucose estimation, if measurement cannot be done immediately.

ABBREVIATIONS

GOD-POD : *Glucose Oxidase-Peroxidase*
SPSS   : *Statistical Package for Social Science*
ANOVA   : *Analysis of Variance*
HSD   : *Honestly Significant Difference*
RBC   : *Red Blood Cell*
ATP   : *Adenosine Tri Phosphate*

1. INTRODUCTION

Diabetes risk assessment and its diagnosis, both requires accurate measurement of glucose concentrations in plasma worldwide. Because of fixed cut-off values, accurate measurements of plasma glucose concentrations are a key factor to classify patients as diabetic; prediabetic, and non diabetic as well as for further course of management.

A number of factors affect the accuracy of glucose estimation. The pre-analytical (i.e. they occur before the sample arrives in the laboratory) loss of glucose from samples during the first 1–2 h after collection is likely a larger source of error, perhaps reflecting a mistaken belief that use of sodium fluoride (NaF) has solved the pre-analytical problems [1].

In many of the government hospitals it is seen that pre-analytical factors such as time interval between collection and separation, time for analysis and temperature is usually not maintained which all can impact a test result. Hence the pre-analytical variations must be reduced to acceptable levels at which they cause no impact on clinical interpretation of the results [2].

The handling and transportation of blood samples collected for glucose analysis has been little studied in recent years. It has been known for many years that the loss of glucose can be prevented by immediately keeping blood-collection tubes on ice, centrifuging the samples with minimal delay in a refrigerated centrifuge, and removing the plasma promptly, but its impractical in modern healthcare [1].

For improving the measurement of glucose, clinical chemists in diagnostic companies and hospital laboratories have made huge advancements, trying to make it as accurate as possible. By contrast, the pre-analytical issues related to glucose measurement are still unsolvable.

Bruns and Knowler [1] hence, in the present study we measured serum glucose concentrations at different temperatures and at different time intervals after collection and at room temperature but separating plasma at different time intervals, to test the assumption that proper storage temperature and time must be considered for plasma glucose estimation, if measurement cannot be done immediately.

2. MATERIALS AND METHODS

2.1 Study Subjects

For this cross-sectional study a total of 17 apparently healthy volunteers aged 20-30 years were enrolled with their consent. Exclusion criteria were age <20 or >30 years, subjects taking treatment of dyslipidemia or hypertension, individuals with history of diabetes, endocrine disorders, kidney diseases, cardiac diseases, any infectious disease in the past two weeks, pregnant or lactating women.

2.2 Blood Samples Collection and Measurement of Analytes

For the biochemical analysis, 12 mL of blood was collected from the antecubital vein in forearm of each volunteer and was divided in six sterile fluoride vacutainers. Out of the six fluoride vacutainers, two of them was allowed to clot at room temperature for 20 min and then centrifuged. The separated sera from these two vacutainers were then divided into 16 parts. Each part was subjected to different temperature (2-8, 30, 35, 40 ± 1 degree Celsius using fridge and laboratory bath) and time interval (2, 4, 6, & 8 hours) before analysis. The left over 4 fluoride vacutainers were kept at room temperature for 2, 4, 6, & 8 hours and then centrifuged and analyzed. Plasma glucose levels were measured in the laboratory of Department of Biochemistry, Center for Basic Sciences, KMC Bejai, Mangalore.

Glucose in plasma was measured with glucose oxidase-peroxidase (GOD- POD) enzymatic method by STAR 21 Plus semiautoanalyser using commercially available kit provided by ERBA diagnostics Mannheim GmbH. The results were expressed in mg/dL.

2.3 Statistics

Data were analyzed using Statistical Package for Social Science (SPSS), version 17.00 (IBM SPSS statistics). Results are presented as Mean ± Standard deviation. Data analysis was done using ANOVA, Bonferonni 't' test and Tukey HSD. The results were considered significant if $p < 0.05$.

3. RESULTS

3.1 Comparison of Mean Plasma Glucose at Different Temperatures and at Different Time Intervals after Collection of Blood and Immediate Separation of Plasma with 0 hr Value

The mean of plasma glucose of 0 hr was 78.16 ± 13.28 mg/dL. The value of plasma glucose decreases significantly at different temperatures and at different time intervals (Table 1).

3.2 Comparison of Mean Plasma Glucose at Room Temperature but Separating Plasma at Different Time Intervals after Collection with 0 Hour Value

The value of glucose decreases significantly with delayed separation of plasma (Table 2).

3.3 Changes in Mean Plasma Glucose with Storage Temperatures, Time Intervals and Time Interval for Plasma Separation

Fig. 1 shows the decrease in mean plasma glucose as the storage temperatures of plasma sample increases before estimation of plasma glucose at different time intervals whereas Fig. 2 shows that the mean plasma glucose decreases as the time interval for plasma glucose estimation increases from the time of blood collection at different temperatures. Also the mean plasma glucose decreases even at room temperature if the time interval for plasma separation increases from the time of blood collection as shown in Fig. 3.

4. DISCUSSION

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underutilized, producing hyperglycemia. The National Academy of Clinical Biochemistry has developed evidence-based guidelines for the practice of laboratory medicine. Little increase in blood glucose significantly increase the risk of developing diabetes mellitus; but pre-analytical and analytical variables make it difficult to correctly apply these epidemiological insights to individual patients [3].

Human mistakes are largely responsible for pre-analytical errors [4] and the majority of these errors are escapable [5]. This is reasonable, since human handling is much more in pre-analytical phase as compared to the analytical and post-analytical phases [4].

Measurement of plasma glucose remains the sole diagnostic criterion for diabetes. The alterations in glucose levels during transport and storage have been observed through years in the laboratories. The pre-analytical issues surrounding glucose estimations remain unresolved till date with only little information

Table 1. Comparison of mean plasma glucose (mg/dL) at different temperatures and at different time intervals after blood collection and immediate separation of plasma with 0 hour value

Time Interval Temperature	Mean plasma glucose at 0 Hr – 78.16 ± 13.28 mg/dL			
	2 hour	4 hour	6 hour	8hour
2-8°Celsius	71.57 ± 12.64	56.44 ± 8.54	54.76 ± 8.48	42.93 ± 8.05
(p value)	(0.696)	(<0.001)	(<0.001)	(<0.001)
30°Celsius	66.49 ± 12.42	50.78 ± 8.31	48.99 ± 8.36	34.30 ± 8.24
(p value)	(0.015)	(<0.001)	(<0.001)	(<0.001)
35°Celsius	61.66 ± 11.79	46.77 ± 7.95	45.07 ± 7.76	30.48 ± 8.04
(p value)	(<0.001)	(<0.001)	(<0.001)	(<0.001)
40°Celsius	55.63 ± 10.94	42.78 ± 6.45	40.79 ± 6.43	24.05 ± 7.02
(p value)	(<0.001)	(<0.001)	(<0.001)	(<0.001)

Results are shown as Mean ± SD, 0 Hr – represents first value after collection and immediate separation of plasma, p value – when compared with 0 hour value, p<0.05 was considered significant

Table 2. Comparison of mean plasma glucose at room temperature but separating plasma at different time intervals after collection with 0 hour value

Time interval of plasma separation	Mean plasma glucose (mg/dL)	p Value with respect to 0 hour value
0 Hour	78.16 ± 13.28	----
2 hour	69.55 ± 12.99	(<0.001)
4 hour	64.29 ± 9.77	(<0.001)
6 hour	57.02 ± 9.62	(<0.001)
8hour	56.95 ± 10.22	(<0.001)

Results are shown as Mean ± SD, 0 Hr – represents first value after collection and immediate separation of plasma, p value – when compared with 0 hour value, p<0.05 was considered significant
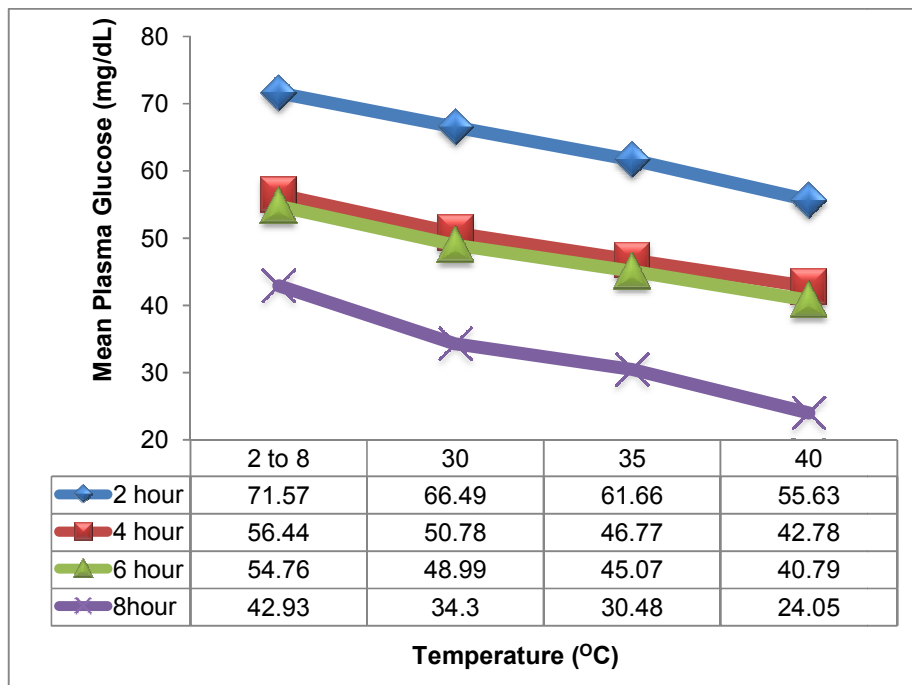

	2 to 8	30	35	40
2 hour	71.57	66.49	61.66	55.63
4 hour	56.44	50.78	46.77	42.78
6 hour	54.76	48.99	45.07	40.79
8hour	42.93	34.3	30.48	24.05

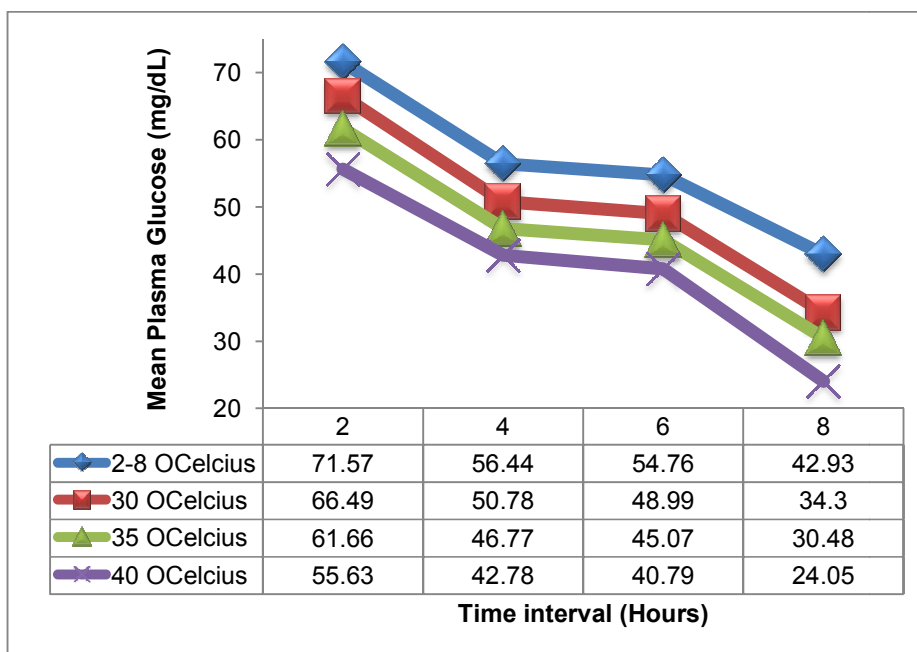**Fig. 1. Means plot showing mean plasma glucose at different temperatures**

Fig. 2. Means plot showing mean plasma glucose at different time intervals
RT- Room Temperature, 0 hr – represents first value after collection and immediate separation of plasma
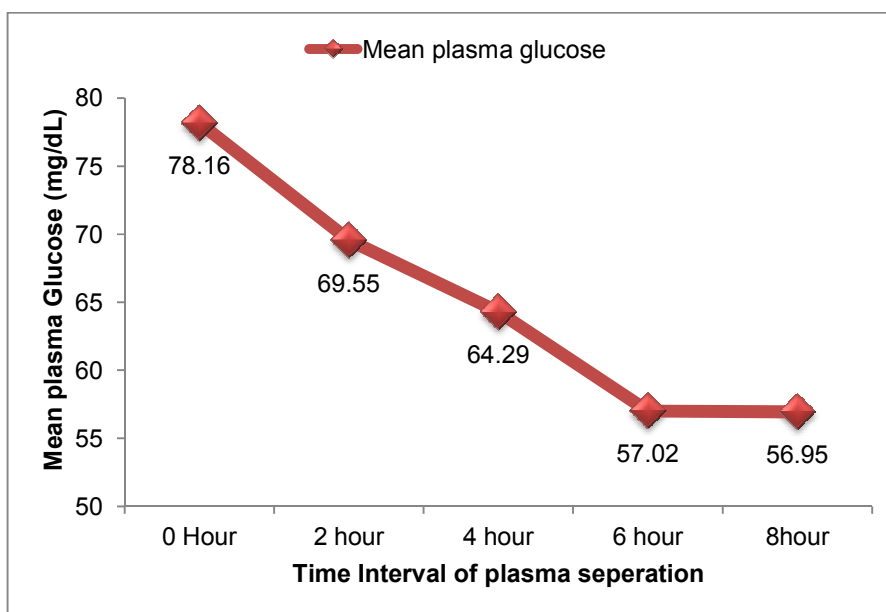

Fig. 3. Means plot showing mean plasma glucose at room temperature but separating plasma at different time intervals
0 hour – represents first value after collection and immediate separation of plasma

available on the stability of glucose during storage and transport [5]. The information on the stability of glucose during storage is often incomplete and sometimes contradictory. In view of these discrepancies, the present study was taken up aiming to identify the quantitative actual alterations in plasma glucose values under the conditions in which the sample reaches the laboratory and analysed by assessing the effect of temperature, time interval for analysis and separation of plasma on glucose levels.

In this study it was observed that glucose should be measured immediately after drawing of blood and separating it from RBC's and if not the sample must be separated immediately and stored at 2-8°C for not more than 2 hrs to prevent erroneous results and reporting.

When blood is collected, erythrocytes and leukocytes do not die immediately and continue to metabolize glucose as a source of energy, via the glycolytic process, thereby decreases glucose concentration [6]. It is been reported that glucose is lost through glycolysis at a rate of 5%–7%/hr. The rate of decrease in plain tubes is sensitive to the clot contact time and temperature [7]. Even in the presence of fluoride, glucose is phosphorylated by available ATP, and the glucose 6- phosphate formed is further metabolized until equilibria are reached in reactions proximal to enolase in the glycolytic pathway [8]. This actually shows that anticoagulants cannot stop, in totality, the breakdown of glucose (glycolysis) [6].

The method of glucose estimation also plays an important role in determining the glucose concentration in plasma. Glucose oxidase (GOD) enzyme in the GOD-POD method of glucose estimation rapidly oxidises beta anomer of D-glucose whereas alpha anomer oxidation is very slow [9,10]. In the serum D-glucose exists mainly in beta form, but on standing, this beta glucose converts into alpha glucose by the process of mutarotation i.e. spontaneous interconversion of beta D- glucose and alpha D- glucose (glucose form in serum). This process of mutarotation is directly proportional to temperature, thereby increasing the proportion of alpha anomer at equilibrium and thus decreasing the proportion of beta anomer resulting in decreased estimated glucose value in plasma by GOD-POD method [11].

Authors also hypothesize that the decrease in glucose concentration in serum at different temperatures may also be due to spontaneous glycation of serum proteins and as the temperature increases the process of glycation also increases. Further studies are required to measure the rate of glycation of proteins and various factors affecting it.

5. CONCLUSION

In conclusion, plasma glucose should be analysed in samples as early as possible after collection of blood, and if not possible, plasma for glucose estimation should be separated immediately after collection of blood and should be stored at 2-8 degree Celsius for not more than 2 hrs for analysis.

CONSENT

All authors declare that written informed consent was obtained from the volunteers for publication as per international and university standards.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bruns DE, Knowler WC. Stabilization of glucose in blood samples: Why it matters. Clin Chem. 2009;55(5):850–52.
2. Marjani A. Effect of storage time and temperature on some serum analytes. Internet J. Lab Med. 2006;2(2).
3. Sacks DB, Bruns DE, Goldstein DE, Maclaren NK, McDonald JM, Parrot M. Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clinical Chemistry. 2002;48(3):436–72.
4. Wiwanitkit V. Types and frequency of preanalytical mistakes in the first Thai ISO 9002:1994 certified clinical laboratory, a 6 - month monitoring. BMC Clin Pathol. 2001;1:5.
5. Carraro P, Plebani M. Errors in a stat laboratory: Types and frequencies 10 years later. Clin Chem. 2007;53:1338-42.
6. Spencer NCO, Sunday JJ, Kingsley O, Uche OC, Adeniyi SA, Chi-Nenye O. Effects of anticoagulants on fasting blood glucose of diabetics and non-diabetics individuals, as well as random blood glucose of apparently healthy individuals, determined by one touch ultra glucometer. International Journal of Pharmaceutical and Clinical Science. 2012;2(4):11-13.

7. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, Bruns DE, (Ed). Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th Edition, USA. Elsevier Inc. 2006;868-9.

8. Li G, Cabanero M, Wang Z, Wang H, Huang T, Alexis H, Eid I, Muth G, Pincus MR. Comparison of glucose determinations on blood samples collected in three types of tubes. Annals of Clinical & Laboratory Science. 2013;43(3):278-84.

9. Keston AS, Brandt R. Method for analysis of the anomers of glucose. Analytical Biochemistry. 1963;6(5):461-7.

10. Keilin D, Hartree EF. Specificity of glucose oxidase (notatin). Biochemical Journal. 1952;50(3):331.

11. Le Barc'H N, Grossel JM, Looten P, Mathlouthi M. Kinetic study of the mutarotation of D-glucose in concentrated aqueous solution by gas-liquid chromatography. Food Chemistry. 2001;74(1):119-24.