Original Research Article

Effect Of Time Interval Between Collection, Plasma Separation And Analysis And The Effect Of Temperature On The Laboratory Results Of Plasma Glucose Estimation.

ABSTRACT

Introduction: Reliability cannot be achieved in a clinical laboratory through the control of 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 wereenrolled with their consent. Plasma glucose levels were estimated by glucose oxidase- peroxidase kit method in 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 2hrs when stored at 2-8 ^{o}Celcius after plasma separation(71.57 ± 12.64), non- significant difference was noted(pvalue – 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.

Keywords: Pre-analytical errors, glucose estimation, separation, temperature, delay

1. INTRODUCTION

Measurements of glucose are used worldwide to diagnose diabetes and to identify patients at risk of developing diabetes. For both diagnosis and risk assessment, fixed cut-off points

of plasma glucose concentrations are used to classify patients and to make decisions regarding 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]. ~~[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]. ~~[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]. ~~[1]~~

Clinical chemists in hospital laboratories and diagnostic companies have made great strides in improving the measurement of glucose. By contrast, the pre-analytical issues surrounding glucose measurements have not been solved[1]. ~~[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. MATERIAL AND METHODS

2.1 Study Subjects

For this cross-sectional study a total of 17 apparently healthy volunteers aged 20-30 years wereenrolled 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~~ Measurement of ~~analytes~~Analytes

For the biochemical analysis, 12m~~l~~L 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 was then divided into 16 parts. Each part was subjected to different temperature (2-8, 30, 35, 40 ± 1degree celcius 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 Plussemiautoanalyser 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 0hr value

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

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 ^{O}Celcius	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 ^{O}Celcius	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 ^{O}Celcius	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 ^{O}Celcius	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

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 significantlywith delayed separation of plasma. (Table 2)

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

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

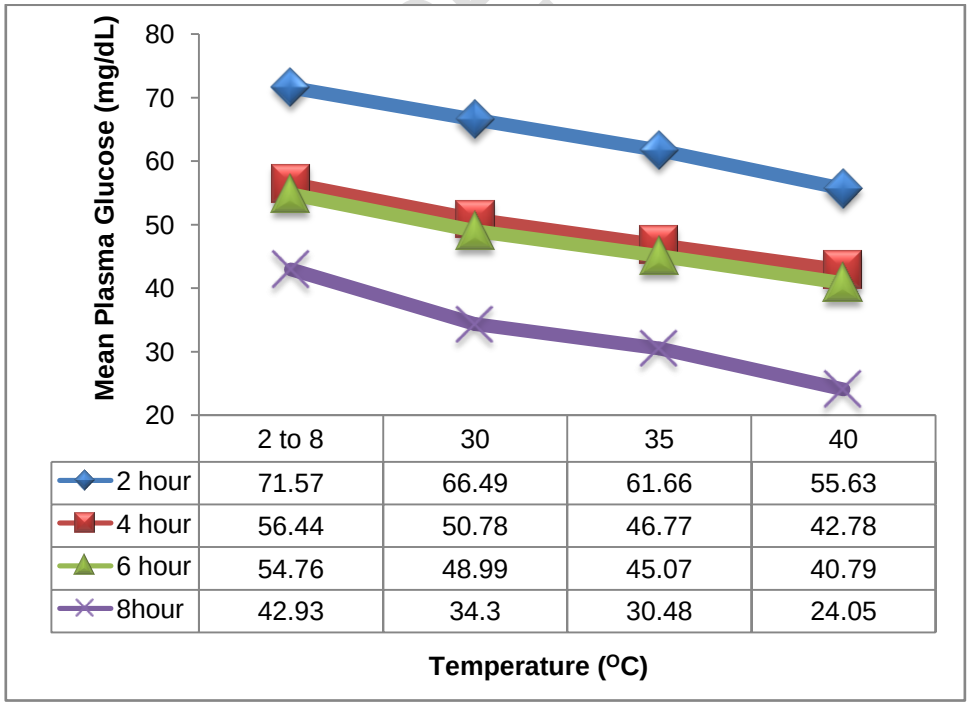
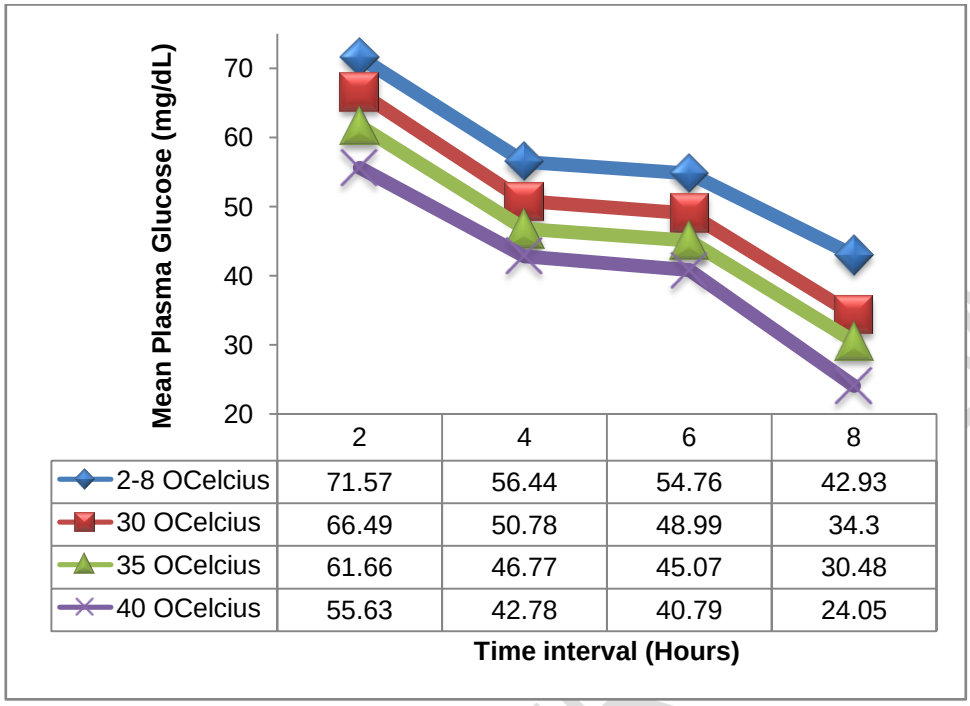


Figure 1: Means plot showing mean plasma glucose at different temperatures

RT- Room Temperature, 0 hr – represents first value after collection and immediate separation of plasma



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

Figure 2: Means plot showing mean plasma glucose at different time intervals

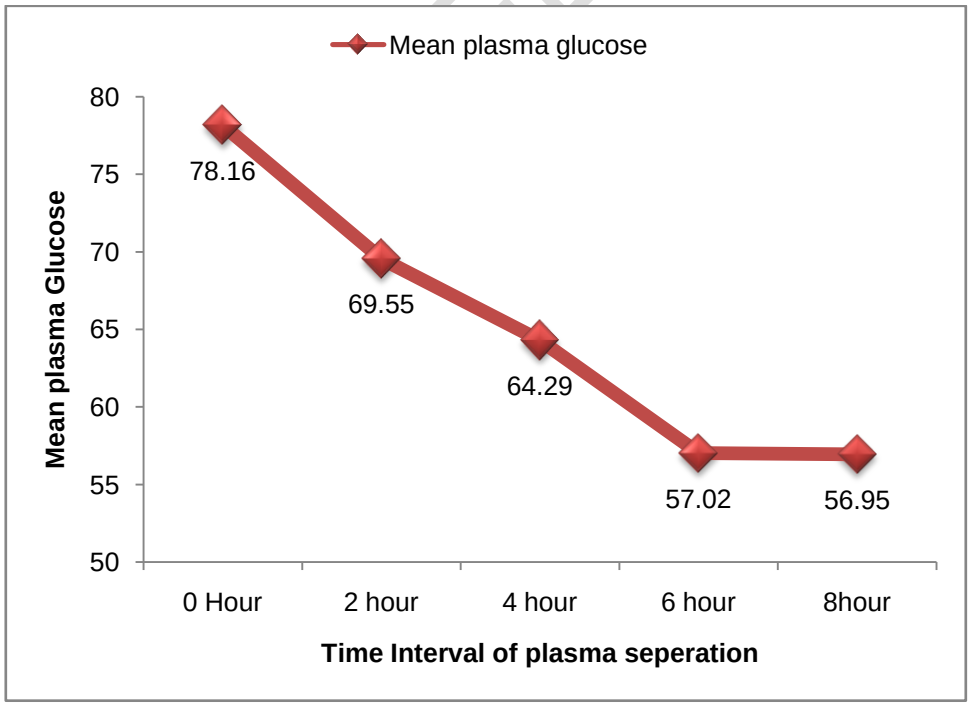


Figure 3: Means plot showing mean plasma glucose at room temperature but separating plasma at different time intervals

0 hour – represents first value after collection and immediate separation of plasma

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. Small increments in blood glucose substantially 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]. ~~[3]~~

Pre-analytical errors are largely attributable to human mistakes [4] and the majority of these errors are preventable[5]. ~~[5]~~ This is understandable, since the pre-analytical phase involves much more human handling, compared to the analytical and post-analytical phases[4]. ~~[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 available on the stability of glucose during storage and transport[5]. ~~[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 alterations in plasma glucose values under the actual 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 ^{O}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]. ~~[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].~~[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]. ~~[8]~~ This actually shows that anticoagulants cannot stop, in totality, the breakdown of glucose (glycolysis)[6].~~[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 celcius 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.

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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