

Original Research Article

THE IMPACT OF DELAYED SEPARATION OF SAMPLE ON GLUCOSE PARAMETER OF APPARENTLY HEALTHY STUDENTS OF FEDERAL SCHOOL OF MEDICAL LABORATORY TECHNOLOGY (SCIENCE), JOS, NIGERIA

ABSTRACT

Background: Estimation of glucose is very important in clinical diagnosis of diabetes mellitus, hyperglycemia and hypoglycemia. The core aim of clinical laboratory service is to produce accurate, precise and reliable test results. The ability of laboratories to do this lies in the availability of well trained personnel, equipment, electricity etc. There has been a progressive increase in the prevalence of diabetes mellitus in Nigeria and the burden is expected to increase even further and the need for proper diagnosis cannot be overemphasized. **Aim:** Here, we designed an experiment aimed at evaluating the impact of delayed separation of plasma and serum from cells and clot on glucose level. **Method:** A total of fifty (50) randomly selected apparently healthy students of Federal School of Medical Laboratory Technology (Science), Jos were recruited as subject into this study. Blood samples were collected from each of the subjects into plain and anticoagulant bottles for serum and plasma respectively. An aliquot was analyzed within 10 minutes to obtain a baseline value where other values after timely delays were compared. The samples were spun but not separated from the cells/clot all through the period of the analysis. Blood glucose was determined by glucose Oxidase Colourimetric assay kit obtained from Randox Laboratories Limited United Kingdom. Data were analyzed using student's t-test and performed using the Statistical Package for Social Sciences (SPSS) version 20.0. **Results:** Result obtained shows the mean and standard deviation of all timing for plasma and serum. In plasma it shows that the means \pm SD of 2, 4 and 24hours (5.2 \pm 0.7, 5.1 \pm 0.4 and 5.3 \pm 0.5 respectively) were not significantly varied when compared to the baseline (0 hour) value (5.3 \pm 0.5). That of baseline and 24 hours (5.3 \pm 0.5 and 5.3 \pm 0.5 respectively) were significantly higher ($P < 0.001$) than that of 48 and 74 hours (1.0 \pm 0.20 and 7 \pm 0.2 respectively). In serum, similar results were obtained. **Conclusion:** In conclusion, where possible, it is advised that the established best practices in processing and analysis of samples be adhered to. However, the results of this work shows reasonably stable results for blood glucose determination could be obtained within 24 hours. Therefore, instead of discarding the sample, it might seem more appropriate that the sample be analyzed and the result reported indicating the number of hours delayed within 24hours.

Key words: Preanalytical Error, Blood Glucose, Diabetes Mellitus, Hyperglycemia, Delayed Sample Separation

INTRODUCTION

Estimation of glucose is very important in clinical diagnosis of diabetes, hyperglycemia, hypoglycemia and normoglycemia [1]. The core aim of clinical laboratory service is to produce accurate, precise and reliable test results. The ability of laboratories to do this lies in the availability of well trained personnel, equipment, electricity etc. In Nigeria for instance, it is not arguable that there is a good number of well trained Medical Laboratory Personnel to man our medical laboratories but these personnel are grossly unemployed or under employed leading to their mass movement abroad for greener pasture and leaving the few employed ones overwhelmed with work load in the laboratories with the attendant delay in turnaround time, quality of results and poor health outcomes [2]. Again, in Nigeria as well as other resource deficient countries there is still a big challenge of no/poor electricity in many urban, sub-urban and rural settlements and lack of essential laboratory equipment such as centrifuge in many sub-urban and rural areas therefore making timely processing and testing of samples near impossible in those areas [3]. Again, the use of satellite collection centers such as the central collection centers in the outpatient departments (OPD) in collecting samples also result in delays of sample separation due to transportation challenges. The results of blood glucose determinations can be strongly affected by the delay in separation of samples which ultimately results in the delay in the analysis [4,5].

Background of the Study

There has been a progressive increase in the prevalence of diabetes mellitus in Nigeria and the burden is expected to increase even further. Diabetes mellitus is a metabolic disease that results from absolute or relative deficiency of insulin, characterized by persistent increase in blood glucose levels above normal, termed hyperglycemia and its attendant complications [6,7]. Blood glucose test is usually done to diagnose and or manage diabetes mellitus. The sample types for blood glucose include Fasting Blood Sugar/glucose (FBS), Random Blood Glucose (RBS), 2 hours Post Prandial and Oral Glucose Tolerance Test (OGTT) [8,9]. The two most common forms of diabetes are type 1 and type 2. Type 1 diabetes results from the autoimmune destruction of the pancreas's beta cells, which produce insulin [10]. Type 2 diabetes is mainly caused by a combination of insulin resistance and relative insulin deficiency. A small proportion of diabetes cases might be types other than type 1 or type 2, such as maturity-onset diabetes of the young or latent autoimmune diabetes in adults [10].

The burden of diabetes is growing in Africa. It is estimated that the African region will have the greatest percentage increase (143%) in the burden of diabetes between 2019 and 2045. Similarly, there has been a progressive increase in the prevalence of diabetes mellitus in Nigeria. Studies have reported prevalence rates of diabetes mellitus ranging between 0.8-4.4% in some rural communities of Nigeria, while the prevalence in urban areas has been reported to range between 4.6-7%. A recently published systemic review and meta-analysis of studies on the prevalence of

diabetes mellitus amongst Nigerians has reported an overall pooled prevalence of 5.77%. As at 2019, 8.2 million Nigerians were estimated to have impaired glucose tolerance, with the number projected to increase to 11.5 million by 2030 [4-10].

Since blood glucose test is very useful in the diagnosis of diabetes mellitus [11,12], it is very important that laboratories produce accurate and timely results for proper and timely management of the patients. The results of blood glucose determinations can be strongly affected by the method of storage and handling of the blood samples between the time of collection and the time of analysis. The major challenge faced by clinical laboratories worldwide is ensuring the integrity and reliability of laboratory results but as analytical variations are being reasonably minimized by the development of new techniques, the relative contribution of pre-analytical factors to spurious results has become a more dominant element in overall test variability [13]

A preanalytical factor of great importance in glucose testing is glycolysis *ex vivo*. According to the recommendation of the World Health Organization [14], the diagnostic sample for blood glucose determination must be venous plasma placed in ice-water slurry after collection, and separation from cells needs to be done before 30 minutes [14,15], otherwise, glycolysis will cause decreased glucose results with respect to their *in vivo* value. Elimination of glycolysis is therefore said to be essential in order to obtain reliable glycaemic results for diagnosis, since it introduces an unpredictable negative bias [16]. Therefore standard operating procedures have been adopted and this is that plasma glucose estimation should be performed within two hours after the collection of sample, and sample preparation (separation of plasma from cells) for the analysis should be performed within one hour of collection [17].

In Nigeria, our laboratories are constantly faced with the problems of inconsistent power supply such that specimens for analysis are left on the laboratory bench at ambient temperature for several hours or even days before light is restored or alternative source of power is gotten for the analysis to be done [18]. In this study, it was aimed that the impact of delayed separation of plasma and serum from the cells and clot on the glucose level of the samples would be clearly determined.

Methods

Study Design

A total of fifty (50) subjects who were apparently healthy students of Federal School of Medical Laboratory Technology (Science) (FSMLT), Jos were recruited into this study. Fasting blood samples were collected from each of the subjects into plain and Fluoride Oxalate bottles for serum and plasma extraction respectively. An aliquot was analyzed to obtain a baseline value and this represents the value at 'zero' hour where other values after timely delays were compared.

Subject Selection

Subjects were randomly selected among the volunteer students of FSMLT, Jos. They were 20 apparently healthy males and 30 apparently healthy females aged between 20 and 30 years. Individuals on any medication were excluded from the study. All blood samples were collected after obtaining written consent from the participants. Blood drawing was performed according to the standard operating procedures.

Ethical Consideration: Ethical approval for the use of human subjects for research was sought and obtained from the research ethics committee of FSMLT, Jos.

Laboratory Analysis

Sample Collection and Preparation

Four milliliters of fasting blood sample was collected from each subject into each of plain and fluoride oxalate bottles for serum and plasma extraction respectively. Samples were spun but were not separated from the cell/clot all through the period of the analysis and were stored at room temperature all through the period of the analysis. Room temperature in Jos the Plateau State Capital, Nigeria in August 2022 where and when then laboratory analysis was done ranged from 62-76 °F or 16.6-24.4°C [19].

Determination of Blood Glucose

Blood glucose was determined by Glucose Oxidase Colourimetric assay kit obtained from Randox Laboratories Limited United Kingdom [20].

Data Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 20.0. Student's t-test was used to study the significance of the difference in the means. P-value of less than 0.05 was considered statistically significant.

Results

Impact of Delayed Separation on Glucose Level of Serum and Plasma Samples

Table 1, Figure 1 and 2 Show the mean and standard deviation of all timing (delayed separation) for plasma and serum. In plasma, 5.3±0.5, 5.2±0.7, 5.1±0.4, 4.6±0.7, 5.3±0.5, 1.0±0.2, 0.7±0.2 were gotten for 0, 2, 4, 6, 24, 48 and 72 hours delays respectively and in serum, 4.4±0.9, 4.7±0.6, 4.5±0.5, 4.3±0.4, 4.4±0.9, 0.6±0.3, 0.4±0.2 were gotten for 0, 2, 4, 6, 24, 48 and 72 hours delays respectively.

Table 2 describes the impact of two and twenty four hourly delayed separation of plasma from whole blood. It shows that the means \pm SD of 2, 4 and 24hours (5.2 \pm 0.7, 5.1 \pm 0.4 and 5.3 \pm 0.5 respectively) were not significantly varied when compared to the baseline (5.3 \pm 0.5). Again, those of baseline and 24 hours (5.3 \pm 0.5 and 5.3 \pm 0.5 respectively) were significantly higher (P <0.001) than those of 48 hours and 74 hours (1.0 \pm 0.20 and 7 \pm 0.2 respectively) and that of 48 hours was significantly higher (1.0 \pm 0.2, P =0.045) than that of 72 hours (0.7 \pm 0.2).

Table 1: Mean and Standard Deviation of All Timing (Delayed) for Both Plasma and Serum

Time of Delay	Plasma			Serum		
	N	Mean	SD	N	Mean	SD
0 hour	50	5.3	0.5	50	4.4	0.9
2 hours	50	5.2	0.7	50	4.7	0.6
4 hours	50	5.1	0.4	50	4.5	0.5
6 hours	50	4.6	0.7	50	4.3	0.4
24 hours	50	5.3	0.5	50	4.4	0.9
48 hours	49	1.0	0.2	50	0.6	0.3
72 hours	49	0.7	0.2	50	0.4	0.2

Result reported as Mean \pm Standard Deviation, N: Number of samples, *Significant at p \leq 0.05, Unit of measurement of glucose is in mmol/L

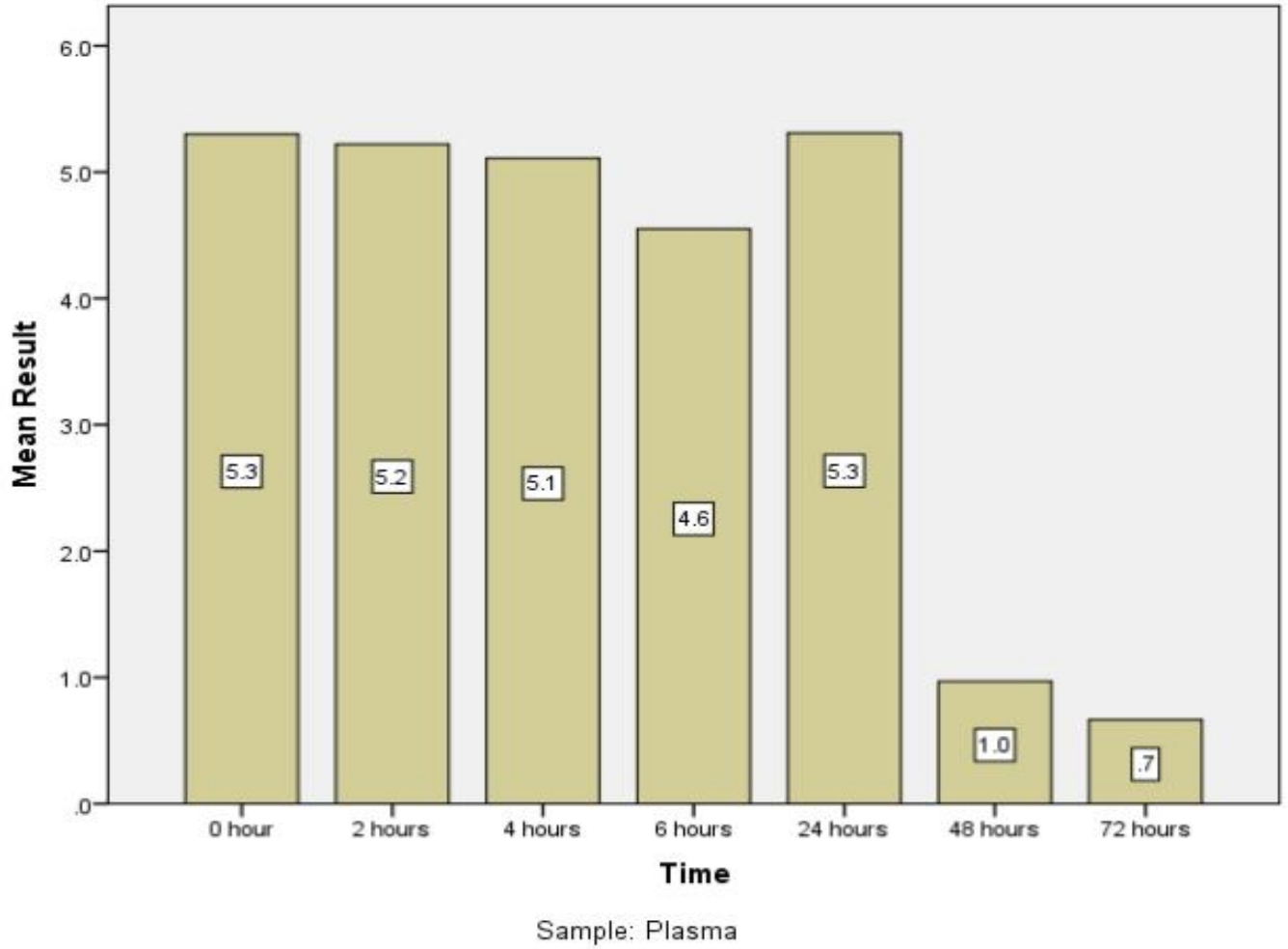


Figure 1: Bar chart of the mean values of the timed delays in plasma samples

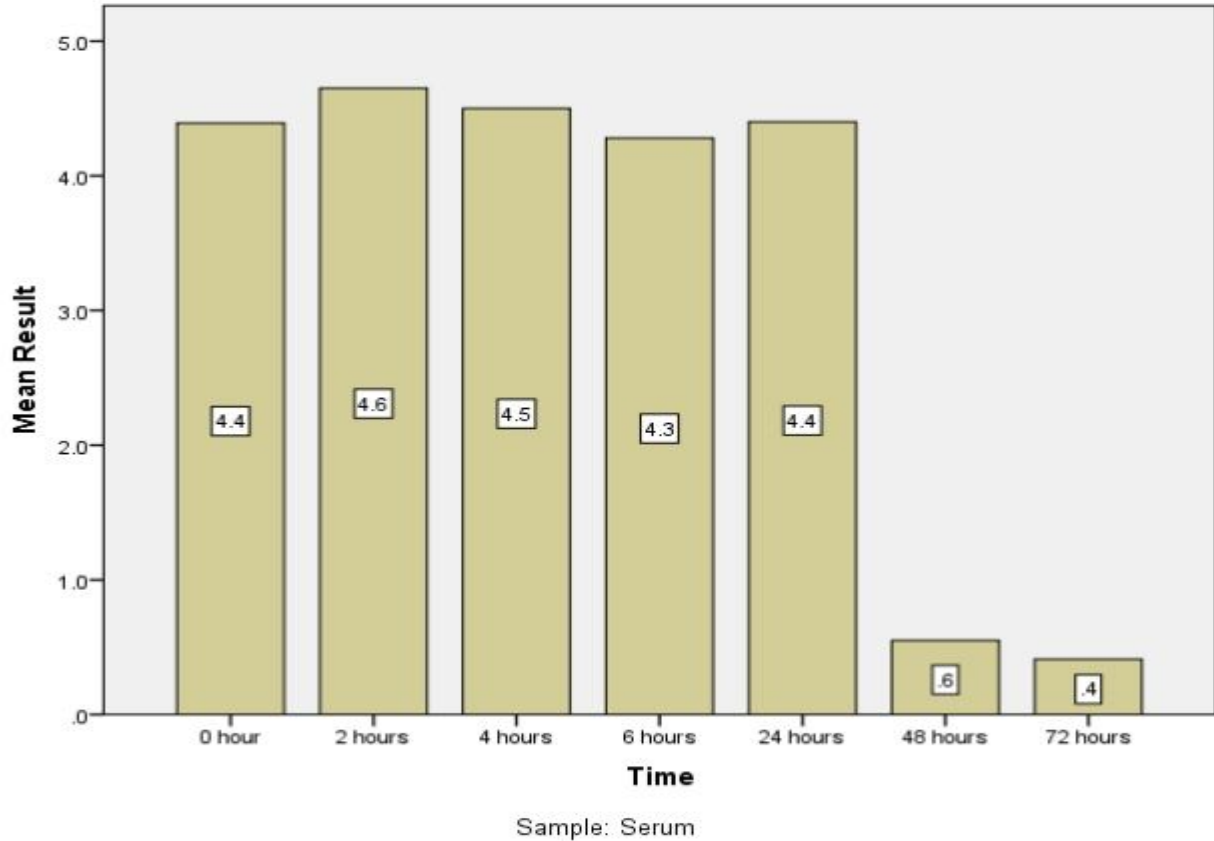


Figure 2: Bar chart of the mean values of the timed delays in serum samples

Table 2: Timed Delay Comparison in Plasma Sample

Time of Delay	Mean 1	Mean 2	<i>p</i> value
0 hour (Mean 1) & 2 hours (Mean 2)	5.3±0.5	5.2±0.7	0.985
2 hours (Mean 1) & 4 hours (Mean 2)	5.2±0.7	5.1±0.4	0.929
4 hours (Mean 1) & 6 hours (Mean 2)	5.1±0.4	4.6±0.7	<0.001*
0 hour (Mean 1) & 24 hours (Mean 2)	5.3±0.5	5.3±0.5	1.000
24 hours (Mean 1) & 48 hours (Mean 2)	5.3±0.5	1.0±0.2	<0.001*
48 hours (Mean 1) & 72 hours (Mean 2)	1.0±0.2	0.7±0.2	0.045*

Result reported as Mean±Standard Deviation, *Significant at $p \leq 0.05$, Number of samples for plasma= 49, others = 50. Unit of measurement of glucose is in mmol/L

Table 3 describes the impact of two and twenty four hourly delayed separation of serum from clotted blood. It shows that there was no statistical significant variation in the means \pm SD of 2, 4, 6 and 24hours (4.7 \pm 0.6, 4.5 \pm 0.5, 4.3 \pm 0.4 and 4.4 \pm 0.9 respectively) compared to the baseline (4.4 \pm 0.9). There was statistically significant variation in the means \pm SD when 48 and 72 hours (0.6 \pm 0.3 and 0.4 \pm 0.2 respectively) were compared with the baseline and 24hours (4.4 \pm 0.9 and 4.4 \pm 0.9 respectively), (P <0.001).

Table 3: Timed Delay Comparison in Serum Sample

Time of Delay	Mean 1	Mean 2	<i>p</i> value
0 hour (Mean 1) & 2 hours (Mean 2)	4.4 \pm 0.9	4.7 \pm 0.6	0.320
2 hours (Mean 1) & 4 hours (Mean 2)	4.7 \pm 0.6	4.5 \pm 0.5	0.875
4 hours (Mean 1) & 6 hours (Mean 2)	4.5 \pm 0.5	4.3 \pm 0.4	0.530
0 hour (Mean 1) & 24 hours (Mean 2)	4.4 \pm 0.9	4.4 \pm 0.9	1.000
24 hours (Mean 1) & 48 hours (Mean 2)	4.4 \pm 0.9	0.6 \pm 0.3	<0.001*
48 hours (Mean 1) & 72 hours (Mean 2)	0.6 \pm 0.3	0.4 \pm 0.2	0.907

Result reported as Mean \pm Standard Deviation, *Significant at $p \leq 0.05$. Unit of measurement of glucose is in mmol/L

Discussion: This study was done to determine the impact of delayed separation of sample on the concentration of glucose in serum and plasma samples. The present study observes that even though it is the best practice to separate sample from cells as soon as it is clotted (for serum) and as soon as it is collected (for plasma), glucose measured from unseparated sample at room temperature could give a reasonable insignificantly varied result within twenty four hours. There may be some unavoidable situation that leads to delay/inability to separate and analyze the sample. Unavailability of the equipment and electricity as is obtained in some rural settings and hard to reach areas, equipment damage or spoilage may be some of the reasons for delayed separation and analysis of samples.

The outcome of this study shows clearly a remarkable reduction in the concentration of glucose in the sample delayed to be separated beyond twenty four hours. Surprisingly, it is one of the few studies that shows that the glucose could be stable in the samples (both plasma and serum) left unseparated within twenty four hours and this is indicated by the insignificant variation in the levels of the analyte when 0, 2, 4, 6, and 24 hours were compared. Effect of delay in Analysis on Plasma Glucose Concentration was recently studied by [21] and reported a statistically

significant change in glucose concentration between the timepoints of one and five hours and three and five hours, however, inconsistent with this present study.

A previous study, [22] stated that delayed separation is one cause of the lack of reproducibility of OGTTs but failed to clearly determine the extent of delays that cause a significant change. Again [23] in their study reported a result inconsistent with this present study, that the decline in serum glucose concentration for all samples stored at 25°C was found to be statistically significant with the Mean glucose value of 1 hour statistically different from baseline value and that at 25°C, serum glucose concentration was found to be decreasing with mean of 10.8% per hour. One of the studies, [24] observed that delays of 15 min or more in analysis reduce clinical accuracy below the ISO 15197:2003(E) recommendation of 95% and that in their study the accuracy was less than 65% for delays of 60 min.

The relative stability of the glucose in these samples up to 24 hours as is reported in this study is not similar with the results of the study of the other authors [21-24] as stated above but seems to be in line with findings of [25]. The relative low atmospheric (room) temperature in the study location which sometimes are as low as the 17°C among other things may be responsible for the stability of the glucose in the sample up to 24 hours. Low temperature is known to slow down glycolysis ex-vivo. The significant reduction of the glucose level when samples were delayed beyond 24 hours may not be unconnected with glycolysis ex-vivo and the fluctuation in the room temperature over and above the course of the 24 hours. This position appears to be agreed by [25].

Conclusion: In conclusion, where possible, it is recommended that the established best practices in processing and analyzing samples be adhered to strictly. Where it is not possible, based on the results of this work, reasonably stable results for blood glucose determination could be obtained within 24 hours especially in areas of low atmospheric temperatures and no sample should be used for analysis when delayed beyond 24 hours as there would be gross reduction of the glucose level. Therefore, instead of discarding the sample, it might seem more appropriate that the sample be analyzed and the result reported indicating the number of hours delayed.

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