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The Impact of Delayed Analysis on Glucose Levels of Blood Samples

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Abstract:- Background: In a hospital setting, glucose is often measured from venous blood in the clinical laboratory. However, laboratory glucose measurements are typically not available in real time. In practice, turn-around times for laboratory measurements can be minutes to hours. This analysis assesses the impact of turn-around time on the effective clinical accuracy of laboratory measurements. Fasting Blood samples was collected from 50-apparently healthy subjects of Federal college of medical laboratory Sciences, Jos, Plateau State, Nigeria. The blood samples were collected into a plain tubes and fluoride oxalate tubes for serum and plasma respectively. Blood glucose estimations was analyzed using Oxidase--Peroxidase method and the Statistical data were analyzed using statistical product and service solution (SPSS) or IBM SPSS version 25.0.The mean ±SD of plasma and serum at baseline of 0-hour,2-hours,4-hours,6-hours,24-hours,48- hours and 72-hours were obtained as 4.63±0.660,4.72±0.368, 5.24±0.440,5.08±0.379,4.77±0.383,4.72±0.442,4.28±0.587 and 4.54± 0.650,4.68±0.655, 4.52±0.535,4.80± 0.653, 4.50±0.631,4.04±1.052,3.77±0.981 for plasma and serum respectively. P≤0.05 was regarded as statistically significant. Finally, the novelty of our study is assessing the effects of time variations on blood glucose concentrations, with this study, we have been able to establish that, there is no stability of glucose concentrations if the collected samples are kept beyond reasonable time of beyond 4 - 6 hours before the analysis is carried out. This report is also in agreement with the research reports of Ambade.

I. INTRODUCTION

Diabetes mellitus is a non-communicable metabolic disease characterized mainly by hyperglycemia (American Diabetes Association,2018). Currently, it has become a common public health problem in the world as well as in Nigeria. Presently, the prevalence of diabetes mellitus in Nigeria is estimated at 37.3 million adults,28.7 million were diagnosed and 8.5 million were undiagnosed. International Diabetes Federation has predicted that diabetes will affect 642 million people worldwide in 2040.

At present, plasma glucose is one of the most common diagnostic tests in clinical laboratories (Weinzimer et al,2003).Out of the total workload in Chemical Pathology Laboratories,30%–40% are related to the estimation of glucose concentration (Nkereuwem et al,2020).Though glycated hemoglobin and plasma glucose testing are both advocated for the diagnosis of diabetes mellitus, the most frequently used primary laboratory test is still the fasting plasma glucose (Kanagazabathy et al,2000).In addition, laboratories receive requests for random plasma glucose, postprandial plasma glucose, and oral glucose tolerance tests. Plasma glucose is analyzed using semiautomated or fully automated analyzers or spectrophotometrically based on the facilities available in a given laboratory. Glucose oxidase-peroxidase and hexokinase are the commonly used enzymatic methods for glucose analysis (Hoedemaekers et al,2012).

Universally, blood is collected to an anticoagulated tube with an antiglycolytic agent for plasma glucose estimation. The current combinations include sodium fluoride/potassium oxalate, buffered sodium citrate, and a combination of sodium fluoride/potassium oxalate or citrate and EDTA for the estimation of the plasma glucose level(Finfer et al,2009).Though glucose can be measured using serum by collecting blood to plain tubes, studies have proven that glucose concentration is higher in plasma than in serum because of the low water content in plasma(International Organization For Standardization,2003).To obtain an accurate value in the estimation of plasma glucose result,it is better to use powder form anticoagulant containing tubes to prevent the dilutional effect of blood by a liquid anticoagulant. According to literature, mixing of 100 µL of liquid sodium fluoride-potassium oxalate in 2.7 mL of blood can cause a 3.7% of dilution (Clarke, et al.1997). However, the most critical

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point in sample collection is the inhibition of in vitro glycolysis in the collection tube.In Nigeria clinical practice, sodium fluoride/potassium oxalate tubes are used as collection tubes for the estimation of glucose concentration. Fluoride binds with inorganic phosphates to form fluorophosphates that bind with magnesium ions in the presence of enolase enzyme(Ichai,2010). Then,it inhibits the action of enolase and therefore inhibits a late step of glycolysis. As a result, the plasma glucose level is stabilized. Though fluoride is an inhibitor, the action is not immediate to inhibit in vitro glycolysis (Gambino et al,2013). When samples are kept for one hour at room temperature,5%–7% of glucose is reduced per hour according to the previous observations (Ambade et al,2017).

When blood samples are left unanalyzed, blood cells continue to metabolize the glucose thereby causing a drastic reduction during a real - time analysis and this may give a false negatives results (Klonoff,2011, Hellman,2012). Therefore, the time of analysis of blood samples after the collection is important and vital to the patients health and proper diagnosis. Sacks et al.2002, reported that, once the blood is drawn, the concentration of glucose levels will continue to decrease because of glycolysis, which will occur in erythrocytes, white blood cells and platelets. The rate of glycolysis in the whole blood is reported to be 5--7% depending on various factors such as glucose concentrations, white blood cells count and hematocrit value (Lee-Levandroski et al,2003).This report is in tandem with the results of this research. However, there are some hindrances that may likely cause a delay in the measurements of blood glucose, some reasons like quantity and frequency of blood samples, bad equipment, power failure and bad roads may limit the possibility of immediate analysis. Under such conditions, it is important to recognize that the blood glucose values can be decreased (Kovatcher et al,2004,2005). Similarly, Stork et al,2005 suggested that, serum may be a better fluid of choice when delay is anticipated in the analysis of blood glucose.

- *There are Some Factors that Increases the Blood Glucose Levels, these Factors Include:*

- Too much food, such as a meal or snack with more carbohydrates than usual, Not being active,
- Not producing enough insulin or being on oral diabetes medications,
- Side effects from other medications, such as steroids or antipsychotic medications, Illness—The body releases hormones to fight the illness, and those hormones raise blood glucose levels,
- Stress, which can produce hormones that raise blood glucose levels,
- Short-or long-term pain, such as pain from a sunburn—The body releases hormones that raise blood glucose levels, Menstrual periods, which cause changes in hormonal levels and Dehydration (Winkelman et al,1994).

- *There are Some Factors that Equally Cause A Decrease in Blood Glucose Levels, these Factors Include:*

Not eating enough food, such as a meal or snack with fewer carbohydrates than usual or a missed meal or snack, alcohol, especially on an empty stomach, too much insulin or oral diabetes medications, Side effects from other medications, more physical activity or exercise than usual—physical activity makes the body more sensitive to insulin and can lower blood glucose(Vanden,2001). Similarly, delayed analysis, delayed separation of serum or plasma from the cells and the subjection of the blood samples to unfavorable temperatures can affect the blood glucose levels in a sample (Weinstein et al,2007).This research work seek to establish the effects of delayed analysis on the blood glucose levels.

II. MATERIALS AND METHODS

- *Study Design*

A total of fifty (50) apparently healthy students of Federal College of Medical Laboratory Technology (Science), Jos. were selected for this study. fasting blood samples were collected from each of the subjects into each of plain tubes and Fluoride Oxalate tubes for serum and plasma respectively. These samples were analyzed to obtain a baseline value at zero (0) hour and then subsequently analysed at 2-hours,4-hours,6-hours,24-hours,48-hours and 72-hours respectively.

- *Subjects Selection*

Subjects were randomly selected among the volunteer students of Federal college of medical laboratory Sciences. They were 30 healthy males and 20 healthy females of ages between 20 and 40years.Volunteers on any medications and those women having their menstrual period were excluded from the study. All blood samples were collected after obtaining written consents.

- *Ethical Consideration:*

Ethical approval for the use of human subjects for research was sought and obtained from the ethics committee of Federal college of medical laboratory Sciences.

- *Samples Collection and Preparations*

5 milliliters of whole blood each was collected from each subject into one plain tube and one fluoride oxalate tube for serum and plasma extraction respectively (Oche et al,2000).

- *Determination of Blood Glucose*

Blood glucose was determined by enzymatic Oxidase-Peroxidase Method using Randox assay kits (Randox).

- *Protocol Set up three test Tubes as follows;*

Table 1 Protocol Set up Three Test

Protein	Blank	Standard	Test

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Precipitate	2.9ml.	2.9ml.	2.9ml
Distilled water	0.1ml	----	-----
Working Standard	----	0.1ml	-----
Test sample	-----	------	0.1ml

Mix well and centrifuge at 2500rpm for 5 minutes (Oche et al,2000).

III. DATA ANALYSIS

The statistical analysis was performed using the Statistical Product and Service Solution (SPSS) or IBM SPSS version 25.0 and dependent t-test was used to compare the difference in the means of the baseline values with the values of the timed delays for determination of significant important, P-values less than or equal to 0.05,($P \leq 0.05$) was considered statistically significant.

- *Results:*

Table 2 Mean±SD Of Plasma And Serum Glucose Levels During Delayed Analysis of 0-Hour,2-Hours,4-Hours,6-Hours,24-Hours,48-Hours and 72- Hours Intervals

Time Intervals	Numbers of Samples (N)	Plasma (Mean±SD)	Serum (Mean±SD)	"T"-Test	P-Values (P≤0.05)	Remarks
Baseline 0- Hour	50	4.63 ± 0.660	4.54 ± 0.650	0.770	0.461	Insignificant
2- Hours	50	4.72 ± 0.368	4.68 ± 0.655	0.264	0.798	Insignificant
4-Hours	50	5.24 ± 0.440	4.52 ± 0.535	9.000	0.000	Significant
6- Hours	50	5.08 ± 0.379	4.80 ± 0.653	2.333	0.045	significant
24-Hours	50	4.77 ± 0.383	4.50 ± 0.631	1.681	0.127	Insignificant
48-Hours	50	4.72 ± 0.442	4.04 ± 1.052	2.489	0.034	significant
72- Hours	50	4.28 ± 0.587	3.77 ± 0.981	1.418	0.190	Insignificant

From the Table 1 above, It is observed that the P- values are statistically insignificant when the mean values of the plasma and serum are compared at the baseline of 0-hour and 2- hours intervals. This implies that, at these times frame, the rate of glycolysis is minimal and does not affect the levels of glucose concentrations in both the plasma and the serum samples (American Diabetes Association,2018).

Conversely, at 4-hours and 6-hours delayed intervals, the P-values were 0.000 and 0.045 which are statistically significant, indicating that the rate of glycolysis is extremely high leading to the loss of glucose as it is being used up by the cells during metabolism(Hellman,2012). The mean concentrations of both plasma and serum at 24-hours and 72-hours intervals are statistically insignificant because the glucose concentration has been utilized by the cells during glycolysis within the 4-hours and 6-hours delayed, making the glucose concentration to be depleted and so having minimal concentration in both plasma and serum(Kilgore et al,1998).The observed Statistical significant of P-value at 48-hours delayed analysis could be due to a systemic errors and the results should be discarded or the test repeated for validity(Howanitz et al,1993).

IV. DISCUSSION

Blood glucose estimations is one of the most common clinical analysis in a clinical laboratories. It is done so as to diagnose an individual of diabetes mellitus or as drugs monitoring therapy (Ibanga et al,2020).

At present, plasma glucose is one of the most common diagnostic tests in clinical laboratories (Ayyaner et al,2018).Out of the total workload in Chemical Pathology Laboratories, 30%–40% are related to the estimation of glucose concentration(Dickson et al,2019).Though glycated hemoglobin and plasma glucose testing are both advocated for the diagnosis of diabetes mellitus, the most frequently used primary laboratory test is still the fasting plasma glucose. In addition, laboratories receive requests for random plasma glucose, postprandial plasma glucose, and oral glucose tolerance tests. Plasma glucose is analyzed using semi-automated or fully automated analyzers or spectrophotometrically based on the facilities available in a given laboratory. Glucose oxidase-peroxidase and hexokinase are the commonly used H7H enzymatic methods for glucose analysis(Carano et al, 2012).

Finally, the novelty of our study is assessing the effects of time variation on blood glucose concentration, with this study, we have been able to establish that, there is no stability of glucose concentrations if the collected samples are kept beyond reasonable time of beyond 4-6 hours before the analysis is carried out. This report is also in agreement with the research report of Ambade et al,2017.

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