e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2024; 16(3); 428-431

Original Research Article

A Lab-Based Assessment of the Blood Glucose Content in EDTA/F Plasma and Serum Samples

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Received: 12-01-2024 / Revised: 18-02-2024 / Accepted: 27-03-2024
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Conflict of interest: Nil

Abstract

Aim: To test the concentration of blood glucose in EDTA/F plasma and serum samples in a clinical laboratory in the Bihar area.

Materials and Methods: This study was conducted in the Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India from Jan 2019 to December 2019. Blood samples for glucose concentration measurements were collected from sixty- three participants irrespective of their age, gender, fasting- and disease state. Written informed consent was obtained from all participants. Different glucose measurements were done for each participant on four occasions based on samples obtained at the same time but collected and handled differently.

Results: The P-Glucose and S-Glucose decreased with time, faster in serum than in plasma. The mean glucose concentration at 30 minutes, 4 hours with early centrifugation, and 4 hours with delayed centrifugation along with the percentage decrease in mean blood glucose concentration and the level of significance in between tubes are shown in table 1. The plasma samples at 30 minutes (P1a) were taken as the reference method and were compared with P1b , S1b, and S2a (table 2). An allowable error zone of ±6% around the Deming regression line was calculated and shown as "funnel-shaped" areas. The zones included 98 %, 98 %, and 63 % of the sample results, in the comparison of P1b, S1b, and S2a to P1a, respectively.

Conclusions: Blood glucose measurement in serum can be accepted if the serum separation via centrifugation is achieved within 30 minutes. The avoidance of an extra amount of blood draw in a separate NaF tube and better turn-around-time are advantages of using serum tubes. It should be mandatory to have a centrifuge at all collection centers to achieve blood separation within a reasonable time.

Keywords: Glucose; Plasma; Serum; Serum separator tubes; Sodium fluoride tubes;

Introduction

The measurement of blood glucose concentration is a fundamental diagnostic and monitoring tool in clinical practice, particularly for managing diabetes mellitus. Accurate glucose measurement is crucial for diagnosing hyperglycaemia and hypoglycaemia, monitoring therapeutic interventions, and preventing complications. Blood glucose can be measured in different types of specimens, including serum, plasma, and whole blood. Among these, serum and plasma are most commonly used. However, the type of anticoagulant used in plasma preparation can influence glucose concentrations. This introduction examines the differences in blood glucose concentrations between EDTA/F plasma and serum, discussing the underlying biochemical principles and clinical implications. [1,2]

Serum and plasma are both derived from whole blood but differ in their preparation and composition. Serum is obtained after allowing blood to clot, followed by centrifugation to remove the clot and cells. Plasma, on the other hand, is prepared by centrifuging blood that has been collected in tubes containing anticoagulants, which prevent clotting. As a result, plasma contains fibrinogen and other clotting factors that are absent in serum. [3]

The type of anticoagulant used in plasma preparation can significantly affect glucose measurements. EDTA (ethylenediaminetetraacetic acid) and fluoride (often sodium fluoride, NaF) are commonly used anticoagulants. EDTA acts by chelating calcium, thus preventing coagulation, while fluoride inhibits glycolysis by inhibiting the

enzyme enolase. The inhibition of glycolysis is particularly important for accurate glucose measurement, as ongoing glycolysis in the blood sample can lead to a significant decrease in glucose concentration over time . [4]

The choice between serum and EDTA/F plasma for glucose measurement has significant clinical implications. Inaccurate glucose measurements can lead to misdiagnosis and inappropriate management of conditions like diabetes. The use of EDTA/F plasma, with its glycolysis-inhibiting properties, provides more accurate and reliable glucose concentrations, particularly in settings where immediate processing of blood samples is not feasible. [5]

Moreover, understanding the differences between these sample types is essential for interpreting laboratory results correctly. Clinicians must be aware of the potential for lower glucose concentrations in serum samples due to glycolysis, which can lead to an underestimation of a patient's glucose levels. This knowledge is particularly relevant for the management of critically ill patients, where accurate glucose monitoring is crucial for outcomes. [6,7]

Materials and Methods

This study was conducted in the Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India from Jan 2019 to December 2019. Blood samples for glucose concentration measurements were collected from sixty- three participants irrespective of their age, gender, fasting- and disease state. Written informed consent was obtained from all participants.

Different glucose measurements were done for each participant on four occasions based on samples obtained at the same time but collected and handled differently. For this, total of 8 ml of venous blood was drawn in two NaF/ Na2EDTA tubes for plasma (P1 and P2) and two serum separator tubes for serum (S1 and S2). All venipunctures were performed by a single experienced phlebotomist to minimize venipuncture bias and were carried out between 7 am and 10 am. The room temperature during blood collection was 26.0 °C (range 23.6–28.6 °C). Visibly lipemic, icteric or haemolyzed samples were not included in the study. The plasma tubes used were BD Vacutainer sodium fluoride/sodium EDTA 13 x 75 mm, 2 ml and the serum tubes used were BD Vacutainer serum separator tube (SST) 13 x 100 mm, 5 ml. P1 and S1 were allowed to clot for 20 minutes and then centrifuged at 1600 × g for 10 min using the Remi Neya4 (Remi Electrotechnics' Ltd, India) centrifuge. Then the glucose concentration was determined (results presented as P1a and S1a) at 30 min after blood collection. The remaining plasma and serum were stored at 4 °C. The glucose concentration was determined again after four hours after bringing the tubes at room temperature (P1b and S1b). The P1b and S1b tubes are mentioned as 4 hours tubes with early centrifugation. P2 and S2 were allowed to clot for four hours at room temperature and then centrifuged after which the P-glucose and S-glucose, respectively were determined (P2a and S2a). The P2a and S2a tubes are mentioned as 4 hours tubes with delayed centrifugation. The samples were collected in the phlebotomy room of this laboratory. Therefore, there was no delay in transportation and no effect on environmental temperature. The glucose concentration was determined spectrophotometrically using Randox Imola auto-analyser (Randox Laboratories Limited UK) by a glucose oxidase- peroxidase method. The quality control of the measurements used two levels of quality control material and conducted by the qualified technical personnel and properly documented. Paired specimens (both serum and plasma) from each participant were analysed using the same lot of reagent, eliminating any lot-to-lot variability in the results. The same technician performed the measurements of all study specimens.

The statistical analysis was performed with Statistical Package for Social Sciences (SPSS v.23). The regression analysis for method comparison was done using the Association of Clinical

Results

The P-Glucose and S-Glucose decreased with time, faster in serum than in plasma. The mean glucose concentration at 30 minutes, 4 hours with early centrifugation, and 4 hours with delayed centrifugation along with the percentage decrease in mean blood glucose concentration and the level of significance in between tubes are shown in table 1. The plasma samples at 30 minutes (P1a) were taken as the reference method and were compared with P1b , S1b, and S2a (table 2). An allowable error zone of ±6% around the Deming regression line was calculated and shown as "funnel-shaped" areas. The zones included 98 %, 98 %, and 63 % of the sample results, in the comparison of P1b, S1b, and S2a to P1a, respectively.

Table 1: Mean blood glucose concentration glucose in various tubes at different times along with difference (when P1a is taken as the ref) and the level of significance among various tubes

Variable [Mean ± Standard deviation (mg/dL)]	Percentage decrease in mean blood glucose (%)	p-value (Wilcoxon signed rank)
S1a versus P1a [97.7 ± 14.8 versus 100.8 ± 16.6]	3.07	<0.001
P1b versus P1a [99.5 ± 17 versus 100.8 ± 16.6]	1.28	<0.001
P2a versus P1a [93.5 ± 17 versus 100.8 ± 16.6]	7.24	<0.001
S1b versus P1a [97.0 ± 15 versus 100.8 ± 16.6]	3.70	<0.001
S2a versus P1a [82.6 ± 18.5 versus 100.8 ± 16.6]	18.05	<0.001

Table 2: Deming regression functions and Pearson correlation coefficient (r)

Variable	Deming Slope ± Sd (mg/dL)	Intercept ± Sd	R
P1b versus P1a	1.02 ± 0.01	- 3.37 ± 1.46	0.99
S1b versus P1a	0.90 ± 0.03	6.12 ± 2.85	0.97
S2a versus P1a	1.12 ± 0.05	- 29.95 ± 4.59	0.95

Discussion

In the present study, we found that once the serum is separated from the red cells and analysed within 30 minutes of collection, the glucose concentration will be stable for at least 4 hours and will be the same as in plasma stored under the same conditions. Though the difference at 30 minutes and four hours was statistically significant, this difference was within ± 6 mg/dL, which is within the standard acceptable range as defined by the United States Clinical Laboratory Improvement Amendments (CLIA) guideline. [8] When centrifugation was delayed for 4 hours, there was a large difference in the glucose concentration between serum and plasma samples. We considered 4 hours delay because we receive blood samples from various collection sites and private clinics and transportation may take considerable time. Sometimes the received samples do not come centrifuged. The difference between early centrifuged (P1a and S1a) and delayed centrifuged (P2a and S2a) samples was significant both statistically and clinically in both plasma and serum, i.e. >± 6 mg/dl. The relative mean decrease was 7.2 % and 18.1 % in delayed centrifuged plasma and serum samples, respectively (Table 1). A similar finding has been reported by the earlier studies.[6,7] It is clear that the laboratories using NaF/Na2EDTA tubes will report slightly higher blood glucose than the laboratories using serum tubes. In our study, when samples from NaF/Na2EDTA tubes at 30 minutes (P1a) were taken as the reference, the glycolysis caused a relative reduction of the S-Glucose at 30 minutes (S1a) of 3.1 % and four hours (S1b) of 3.7 %. However, the glycolysis caused a reduction in P-Glucose at four hours (P1b) of only 1.3 %. The Deming regression of P1b compared with P1a as the reference, showed a regression coefficient of about 1.02 and an intercept of about 3 mg/dL (Table 2). The correlation coefficient (r) was 0.99. In a comparison between S1b and P1a the slope was 0.90 and the intercept was 6 mg/dL with a correlation coefficient of 0.97. Within the measuring interval, this corresponds to a mean difference of 1.9 % which is not clinically significant. Thus, the NaF/Na2EDTA and serum tubes can be used interchangeably for analysis of blood glucose up to four hours if centrifuged at 30 minutes. The regression analysis of NaF tubes at 30 min (P1a) versus SST at four hours with delayed centrifugation (S2a) demonstrated that there is a considerable decrease in blood glucose of 18.1 % in S2a but the correlation coefficient (r = 0.95) is acceptable (Table 2). Statistical and clinical significance may be different quantities, where the latter is related to a difference between two results which might trigger a change in the patient care. For P/S-Glucose this amounts to ±6 % (CLIA) [8] and if shown in a diagram appears as a funnel-like zone surrounding the regression. The comparison between P1b and P1a and S1b and P1a showed that 98 % of the results would be within the zone whereas the corresponding result of the comparison between S2 and P1a was about 63 %. If, however, the allowable error zone was 10 % then 98 % of the results would be found within the zone.

Conclusions

Blood glucose measurement in serum can be accepted if the serum separation via centrifugation is achieved within 30 minutes. The avoidance of an extra amount of blood draw in a separate NaF tube and better turn-around-time are advantages of using serum tubes. It should be mandatory to have a

centrifuge at all collection centres to achieve blood separation within a reasonable time.

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