Journal of Pathology of Nepal (2021) Vol. **11**, 1837 - 1841

Journal of
PATHOLOGY
of Nepal
www.acpnepal.com

Original Article

Blood glucose concentration compared in EDTA/F plasma and serum in a referral clinical laboratory in Nepal

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Keywords:

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

ABSTRACT

Background: Sodium fluoride tubes or serum separator tubes are mostly used for blood glucose estimation in the clinical laboratories of Nepal. The study aimed to investigate the stability of glucose in samples collected in serum separator tubes and sodium fluoride/sodium ethylenediaminetetraacetic tubes by comparing the glucose concentration at 30 minutes and 4 hours collected and handled differently to simulate prolonged sample transport between venipunctures, centrifugation, and measurement.

Materials and Methods: Samples were collected from healthy volunteers into two different serum separator tubes and two different sodium fluoride/sodium ethylenediaminetetraacetic tubes. Glucose concentration was measured at 30 minutes after venipuncture and compared with results from the same samples analyzed at four hours and with the results from tubes centrifuged with a delay of 4 hours. Differences between baseline and respective delayed analyzed glucose values for each tube type were tested using the Student's paired t-test and Deming regression.

Results: When comparing plasma glucose at 30 minutes, glycolysis caused a relative reduction of the glucose concentration in serum at 30 minutes of 3.1 %, which is only slightly less than at 4 hours (3.7 %). This is still substantially more than the reduction in plasma at 4 hours (1.3 %). Surprisingly, the difference between plasma glucose at 30 minutes and serum glucose at 4 hours was only 1.9% which is not clinically significant.

Conclusions: The Na-F/Na2 EDTA tubes and serum separator tubes can be used interchangeably for analysis of blood glucose up to 4 hours if centrifuged within 30 minutes.

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Reveived : October 20th 2020 ; **Accepted** : November 22nd 2020

Citation : Pant V, Gautam K, Pradhan S, Pyakurel D, Shrestha A. Blood glucose concentration compared in EDTA/F plasma and serum in a referral clinical laboratory in Nepal. J Pathol Nep. 2021;11: 1837-41. DOI: 10.3126/jpn.v10i2.32351

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INTRODUCTION

Glucose can be measured in whole blood, serum (S-Glucose), or plasma (P-Glucose), but plasma is recommended for diagnosis.[1] Loss of glucose in collection tubes by glycolysis is a serious problem and is often overlooked in our settings. There are a number of enzyme inhibitor/anticoagulant combinations that are available for the stabilization of glucose in the collection tube. The most commonly used glycolysis inhibitor is sodium fluoride (NaF), which inhibits the enzyme enolase. NaF can be used alone or with anticoagulants such as potassium oxalate, ethylenediaminetetraacetic (EDTA), citrate, or lithium heparin. The recent recommendation for stabilization of blood glucose is the use of tubes containing

DOI : 10.3126/jpn.v10i2.32351

a rapid glycolysis inhibitor, i.e. citrate/EDTA buffer which inhibits the upstream enzymes involved in glycolysis unlike the NaF which inhibits the downstream enzyme and allows glycolysis to continue for a considerable time.[1, 2] However, the choice and use of collection tubes in the clinical laboratory also depends upon the availability and economic aspects.

The original recommendations of the American Diabetes Association (ADA) for the stabilization of blood glucose, indicated the following: (1) immediate centrifugation and separation of plasma from blood cells, (2) immediate cooling of the sample tube in an ice-water slurry, and plasma separation within 30 min from blood draw. Clinical organizations do not recommend the measurement of S-Glucose rather than P-Glucose for the diagnosis of diabetes.[3,4] In Nepal, the newly recommended citrate buffer, NaF and EDTA (acidified mixture) containing tubes are costlier and are unavailable. Thus, we are left with the option of using NaF tubes with EDTA or potassium oxalate and centrifuging the sample immediately for separation of plasma or relying on serum separated under carefully selected conditions.

Serum separator tubes (SST) have been extensively used by the laboratories in Nepal for the estimation of S-Glucose. During the centrifugation process, the separator gel in these tubes liquefies and migrates to form a physical barrier between the serum and the cellular components thus preventing glycolysis by the red blood cells. Various studies have shown that there was no difference in glucose concentrations of blood samples collected in SST and fluoride tubes that were separated within two hours of collection.[5,6,7]

At our laboratory, we are using sodium fluoride/sodium EDTA (NaF/Na2EDTA) tube for the estimation of P-Glucose. There is no uniformity in the use of blood collection tubes in clinical laboratories in Nepal. The purpose of this study is to investigate the stability of glucose in samples collected in SST and NaF/Na2EDTA tubes by comparing the glucose concentration at 30 minutes and 4 hours, collected and handled differently. The estimation of sample glucose in different tubes at a different times from the same sample will help us identify the difference in the result which ultimately guides the clinician.

MATERIALS AND METHODS

A cross-sectional observational study was performed at the Samyak Diagnostic Pvt Ltd Clinical Laboratory based in Lalitpur, Kathmandu. 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. Ethical approval to conduct this study was obtained from the Nepal Health Research Council (Protocol number- 932/2019 P).

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 hemolyzed 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 Elektrotechnik 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-analyzer (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 analyzed 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.

All data sets were tested for normality using the Kolmogorov-Smirnov test and expressed as mean ± standard deviation (SD) if normally distributed and as median if not normally distributed. The comparison was evaluated in a pairwise model, i.e. the Student's dependent test and the Wilcoxon rank test, as appropriate. Deming regression was used to

Table 1: Mean blood glucose concentration in various tubes at different times along with percentage decrease in mean blood glucose (when P1a is taken as the reference) 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

**P1a = Sodium fluoride tube at 30 min, S1a = Serum tube at 30 min, P1b =Sodium fluoride tube at 4 hours with early centrifugation, S1b = Serum tube at 4 hours with early centrifugation, P2a = Sodium fluoride tube at 4 hours with delayed centrifugation, S2a = Serum tube at 4 hours with delayed centrifugation*

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

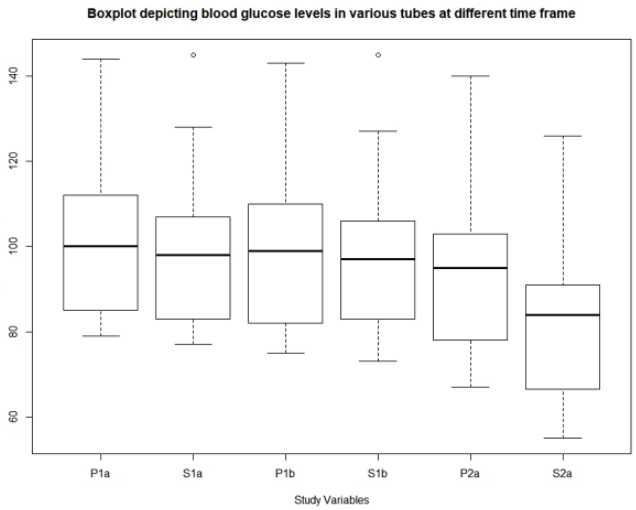

Figure 1: *Mean P/S-Glucose at different times*

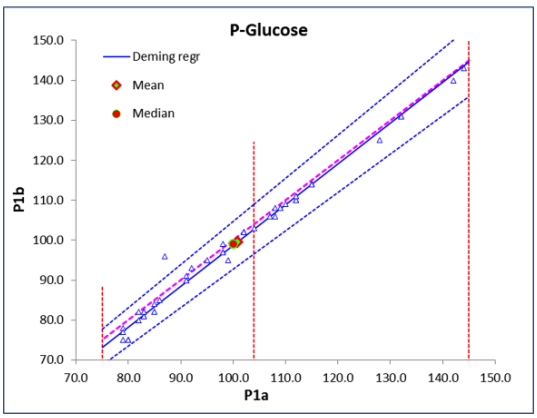

Figure 2- *Deming regression of P-Glucose concentrations at 30 minutes (P1a) and four hours (P1b). The solid line is the regression line and the hatched line is the equal line. The two hatched lines forming a funnel-like entity delineate the allowable error zone, in this case the regression ±6 %. Vertical lines indicate the measurement interval and reference value (104 mg/dL). The average and median values of the cohort are marked.*

compare glucose concentrations in NaF/Na2EDTA tubes and SST assuming the same method uncertainty (variance) for the compared measurements.

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 Biochemistry and Laboratory Medicine (ACB) spreadsheet for verification and method/patient comparison.[8,9] Values of $p<0.05$ were considered statistically significant.

There is a large amount of information in comparisons of sets of data that are not always explored. The ACB spreadsheet automatically presents regression and clinical performance from many different angles, allows the operator to partition the dataset, and presents the Bland-Altman[10] difference graphs in absolute and relative terms.

RESULTS

The P-Glucose and S-Glucose decreased with time, faster in serum than in plasma (fig. 1). The mean glucose concentration at 30 minutes, 4 hours with early centrifugation, and 4

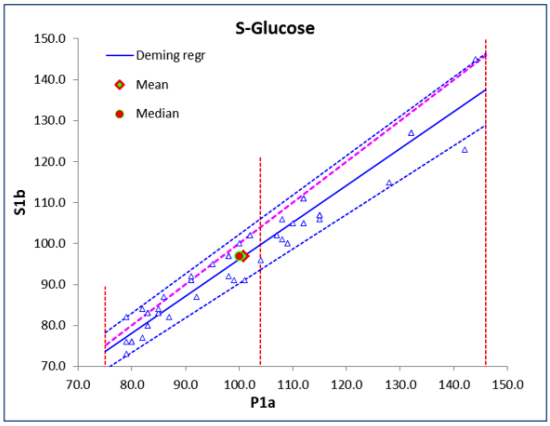

Figure 3: *Deming regression of S-Glucose and P-Glucose concentrations in samples from the same blood draw at 30 minutes (P1a) and four hours (S1b). Symbols as in figure 2.*

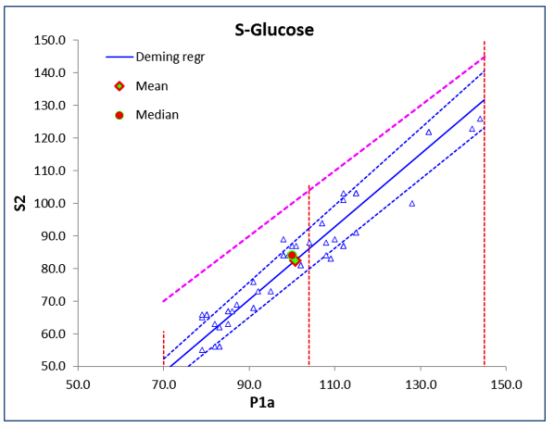

Figure 4: *Deming regression of S-Glucose and P-Glucose concentrations in samples from the same blood draw at 30 minutes (P1a) and four hours (S2a). Symbols as in figure 2.*

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 (fig. 2), S1b (fig 3), and S2a (fig, 4) (table 2). An allowable error zone of ±6% around the Deming regression line was calculated and shown as "funnel-shaped" areas (fig. 2 to 4). The zones included 98 %, 98 %, and 63 % of the sample results, in the comparison of P1b, S1b, and S2a to P1a, respectively.

DISCUSSION

In the present study, we found that once the serum is separated from the red cells and analyzed 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.[11]

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) (fig 4) 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) 11 and if shown in a diagram appears as a funnel-like zone surrounding the regression (fig 2, 3, 4). 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.

DOI : 10.3126/jpn.v10i2.32351

The minimal significant difference can also be calculated as the "MD=minimal difference" or the "RCV = reference change value" [12] using the formulas

MD=k×√(uA2+uB2) and RCV=k×√(uA2+uB2+2×uBio2)

Where k is the coverage factor (conventionally set to 2 to cover 95 % of the variation) and uA, uB and uBio represent the uncertainty of the first, second, and within-individual biological variation, 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.

Footnote

In this report, IFCC-IUPAC notation for measurands was used. Thus, P-glucose stands for plasma glucose concentration and S-glucose stands for serum glucose concentration.[13]

Acknowledgment

Authors would like to thank Dr. Anders Kallner affiliated with the Department of Laboratory Medicine, Karolinska Institute, Sweden for his very generous contribution in the preparation of this manuscript.

Conflict of interest: None

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