A study on the assessment of stability of glucose concentrations in serum separator gel tubes

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Abstract

Introduction: Increasing diabetic burden in India has shifted our health care focus towards its prevention and management of diabetes mellitus. Often multiple investigations are requested by the physicians to assess diabetic status. Conventionally fluoride tubes (glycolytic inhibitor) have been used for glucose analysis in clinical laboratories. Preservatives used here make it unsuitable to measure other key clinical chemistry analytes, for which serum separator gel tubes(SST) are being employed. On centrifugation of SST tubes, a thick gel barrier is formed separating the serum from the cells. This gel barrier prevents utilization of glucose by the cells, hence researchers have considered using it for glucose measurement. This study has been done to assess the integrity of serum samples obtained from SST tubes for analysis of glucose.

Materials and Methods: A single centre cross-sectional study was done, with 140 paired samples collected in fluoride and SST tubes from patients attending Sri Ramachandra Laboratory Services. Samples were analyzed by glucose oxidase-peroxidase method in Siemens ADVIA 1800 Clinical Chemistry at 1 hr, 6 hrs and 24 hrs of sample collection.

Results: Statistical analysis showed no significant change in glucose concentrations measured in fluoride against serum samples. Paired t-test results showed no statistical significance between the tubes, irrespective of time. Bland Altman plot analysis revealed that serum values were not significantly different from fluoride samples throughout the study period. Intraclass correlation (ICC) showed correlation of 0.999 between serum and plasma at 1 hr, 6 hrs and 24 hrs from time of collection.

Conclusions: Serum from SST tubes can be used to measure blood glucose instead of standard accepted fluoride plasma samples.

Keywords: Diabetes mellitus, Fluoride tube, Serum separator gel tube, Analytes, Glucose, Gel barrier.

Introduction

Diabetes mellitus is gaining great attention as an emerging epidemic in developing countries like India. Asians are found to develop the disease at a lower BMI compared to Western population, probably due to decreased skeletal muscle: fat ratio.[1] Surveys show that around 41 million citizens in India have diabetes melliutus and is likely to increase to 70 million by 2025.[2] It is a chronic illness that necessitates a multi-factorial approach involving various risk reduction modalities apart from a stringent glycemic control. Diabetic patients are at a higher risk of developing micro and macro vascular complications like diabetic retinopathy, nephropathy, coronary artery disease etc. The morbidities and mortalities that occur subsequent to these complications can be prevented to a great extent by regular monitoring of their diabetic status.[3,4] Blood glucose levels, HbA1C, renal function tests, lipid profile are done periodically to ensure better patient outcomes.[5,6]

Lots of pre-analytical errors are encountered while measuring glucose in laboratories. When plain tubes devoid of preservatives are used, there is a 5- 7 % decline in glucose values per hour.[7] This decrease is due to ex-vivo glycolysis by the blood cells.[8,9] Sodium fluoride (NaF) inhibits enolase, a glycolytic enzyme, thus reducing ex-vivo glycolysis in the collected blood samples. But fluoride-oxalate generates a shift in osmotic pressure; altering the concentration of analytes reduces the activity of the enzyme urease; and also forms complexes with calcium ions in samples, all of which led to errors in quantifying these analytes in fluoride samples.[10,11] Thus more than one tube is used for blood collection, especially when multiple tests are requested. Meticulous research is being done to minimize the use of multiple blood collection tubes without compromising the quality of glucose concentration in samples.[10,12]

For measurements of blood levels of common clinical chemistry analytes like creatinine, lipid profile, bilirubin, liver enzymes etc, serum specimens are most favored. Serum separator gel tubes (SST) are being employed in clinical laboratories for testing most analytes. SST tubes include a gel which creates a physical barrier between serum and the clot. This unique property of SST has prompted the researchers to explore its use as an alternative tube for blood glucose measurement.

The goal of this study is to compare the glucose concentrations in blood samples collected using SST and fluoride tubes as well as to assess the integrity of glucose concentration in both the tubes over a period of 24 hrs on storage.

Materials and Methods

A single centre cross-sectional study was carried out in patient samples presented to Sri Ramachandra Laboratory Services. The study was approved by Sri Ramachandra University ethics committee. 140 study participants aged more than 18 yrs who attended Sri Ramachandra Laboratory Services in the month of July 2016 were included in the study. Informed consent was obtained. The gray top tube containing sodium fluoride/sodium EDTA 3mg/6mg with 2 ml capacity and yellow top SST tube with 3.5 ml capacity were used for sample collection. Following standard venipuncture protocol, fasting blood samples were collected in one SST and one NaF tube from each participant. Fluoride tubes were centrifuged immediately for 10 min at 4000 rpm to separate plasma. SST tubes centrifuged after 30 min at 4000 rpm for 10 min to separate the serum.

Glucose was quantified using Glucose oxidase-peroxidase enzymatic method in Siemens ADVIA 1800 auto analyzer in both SST and fluoride plasma samples at 1 hr, 6 hrs and 24 hrs of sample collection. Tubes were stored at 2- 4°C between analysis.

Results

All statistical analyses were performed with the Statistical Package for the Social Sciences statistical software package for Windows, version 16.0 (SPSS Inc., Chicago, IL, USA), Bland Altman plot analysis was done using MEDCALC statistical software.

The samples showed glucose values ranging from 77 to 382 mg/dl. Paired t-test was employed to compare glucose concentrations measured in fluoride plasma and SST serum samples. The difference between the 2 tubes at 1 hr (p=0.92), at 6 hrs (p=0.61), and at 24 hrs (p=0.42) were found to be statistically not significant.

Bland Altman plot analysis was performed to show the agreement between plasma and serum glucose at various time intervals(Figures 1A,1B,1C). Analysis of glucose values gave a statistically non-significant constant mean bias of 1 ± 0.003 mg/dl (bias±SE) which demonstrates elevated plasma glucose values regardless of time. A non-significant constant positive bias was also obtained at 6 hr and 24 hr samples.

Results from intraclass correlation (ICC) were found to be 0.999 between serum and plasma at 1 hr, 6 hrs and 24 hrs, showing that plasma and serum values were comparable with good reliability.
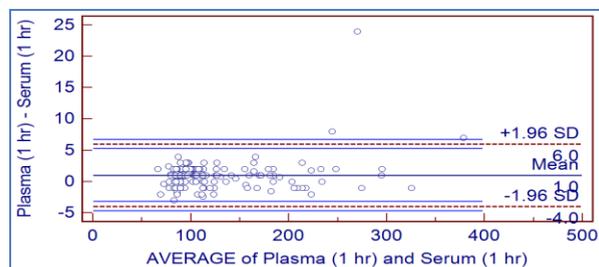

Fig. 1A: Bland Altman plot analysis of glucose values measured in fluoride and SST tubes at 1 hr
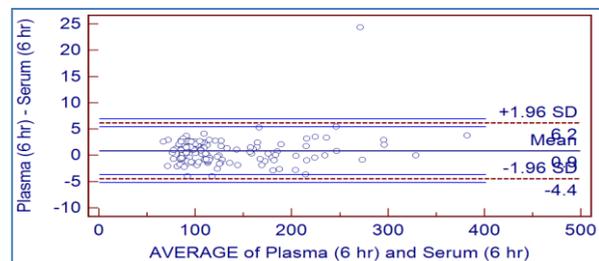

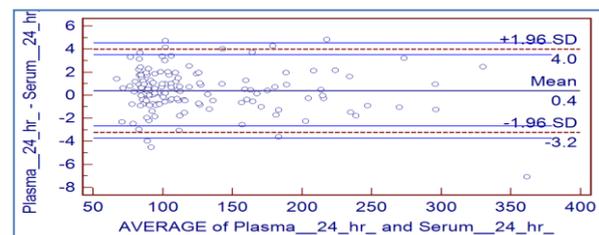**Fig. 1B: Bland Altman plot analysis of glucose values measured in fluoride and SST tubes at 6 hr**

Fig. 1C: Bland Altman plot analysis of glucose values measured in fluoride and SST tubes

To assess the integrity of glucose measurements over time, multiple comparison ANOVA was done. It showed that there is no significant difference in glucose concentrations on storage over a period of 24 hrs (Plasma samples p-value 0.96, SST samples p-value 0.98)

Discussion

In any blood sample, depletion of glucose is an important pre-analytical error, which was minimised by the use of fluoride samples. However, these fluoride samples cannot be used for measuring other analytes like urea,calcium and electrolytes.This has prompted research for alternative tubes for glucose measurement.[11,13]

The progress made in the field of the clinical laboratory has led to the usage of serum separator gel tube for measuring common analytes like creatinine, lipid profile. Serum separator gel tubes (SST) contains a thixotropic gel which forms a layer of barrier gel between serum and blood cells following centrifugation.[14] Advantages are short processing times, can be used directly in measuring analytes in the

instrument, avoidance of aliquoting the samples after analysis as the physical barrier formed by the gel inhibits any contact between blood cells and serum,[15] thus improving the stability of various analytes in the separated serum.[16]

This study reveals that there was no significant variation in glucose measurements between fluoride plasma and serum samples. Therefore the need for a separate fluoride plasma sample for glucose measurement alone appears to be a waste of resources. Studies done by Fernandez et al.[17] and Li et al.[18] have given the same results.

To know the stability of glucose in serum on storage we have measured glucose values at various time intervals (1, 6 and 24 hrs) in both the tubes. The glucose analysis was done at different time intervals directly from the test tube. Statistically significant change was not observed in glucose values during the sample storage time. This demonstrates the stability of gel barrier in SST tubes circumventing the step of aliquoting the serum. The use of SST tubes also offers additional operational benefits, decreases the amount of blood drawn from the patient.

Bland Altman plot analysis demonstrated a non-significant constant positive bias of 1 mg/dl i.e. this infers that plasma values were found to be higher when compared to serum samples of paired specimens. Fernandez et al. in his study verified that there was no evidence of significant variation in glucose values between SST samples and fluoride samples.[17] A study done in 90 paired samples by Gambino et al shows that 0.9% significant increase was seen in fluoride plasma glucose values.[6,11] This discrepancy in the findings can be due to consumption of glucose during the period of clotting of samples.[19] The difference of 1 mg obtained in serum samples will not play a significant role in decision making with respect to treatment of diabetic patients.

Conclusion

The concentration of glucose measured in serum samples (SST tubes) is comparable to that of fluoride plasma samples. The integrity of glucose concentrations is maintained during the storage period of 24 hrs. With increasing number of lab requests for multiple investigations, the use of serum samples for analysis of glucose is clinically acceptable.

Limitations of the study
This study includes glucose values ranging from 77-382 mg/dl. Therefore future studies should be done to cover the entire measurement range of glucose testing procedure.

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