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Are Serum Separator Tubes An Effective Alternative To Sodium Fluoride Tubes For Glucose Estimation?

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ABSTRACT

The stability of glucose in blood samples has been a great challenge in obtaining a confident result that may allow the precise interpretation of glycemic levels, and subsequently the correct management of the diabetic patients. The present study compares the glucose concentrations obtained using different collection tubes (plain vacutainer with no anticoagulants, serum separator tube and sodium fluoride tubes and correlate the glucose values in three different vacutainers at different time intervals. Blood samples were randomly collected in NaF tubes, Serum separator tubes (SST) tubes and plain vacutainers. Glucose concentrations were measured by hexokinase assay. Glucose concentrations were measured at '0' hour (soon after processing) and at 1, 2 and 4 hours of standing at room temperature. Samples were stored in a refrigerator at 4^{o} C and re-assayed after 24 hours. ANOVA was used to find the significant difference in mean glucose levels of samples collected in different tubes. Repeated ANOVA measurement was used to test for difference in glucose levels between different time intervals of study for each of the vacutainers. Mean glucose concentration in SST and Plain tubes were lesser than that in Sodium Fluoride, but not statistically significant. However, at 24 Hours, significant difference was observed in glucose concentration in sodium fluoride, SST and plain tubes. There was no significant difference in mean plasma glucose level in sodium fluoride and SST tubes across time periods. There was a significant difference in mean glucose levels in plain vacutainer across time periods. Present study showed a strong correlation with minimal difference in glucose level between sodium fluoride and SST tubes. The glucose values of sodium fluoride and SST tubes showed a better correlation. Therefore SST tubes can be safely used to estimate glucose levels.

Keywords: Diabetes Mellitus, Serum separator tubes, Sodium fluoride tubes, Plain vacutainers

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INTRODUCTION

Diabetes mellitus is an important health problem affecting major populations worldwide. India is known as the "diabetes capital of the world [1]. The stability of glucose in blood samples has been a great challenge in obtaining a confident result that may allow the precise interpretation of glycemic levels, and subsequently the correct management of the diabetic patients [2]. Red blood cells metabolise glucose via glycolysis resulting in lower glucose concentration of about 7 mg/dl per hour in whole blood [3]. Blood can be collected into red topped tubes with a serum separator gel which sequesters the cells from plasma / serum to prevent this [4]. Use of sample tubes containing sodium fluoride has been established as a means of minimizing ex vivo glycolysis in clinical practice [5]. The Sodium fluoride /Potassium oxalate collection tubes offered the advantage that centrifugation and removal of the plasma from the cellular components could be delayed for several hours without affecting the glucose concentration. These tubes were invaluable for out-patient specimen collection environments as well as field studies where specimen centrifugation immediately post specimen collection was not practical. However, the preservatives in these tubes rendered these specimens inappropriate for measurement of other key analytes such as Na+, K+, enzymes, etc. and as such required the use of multiple different collection containers [6].

The most recent guidelines of American Association of Clinical Chemistry (AACC) and American Diabetes Association (ADA) for laboratory analysis in the diagnosis and management of diabetes mellitus recommend that tubes with only enolase inhibitors, such as sodium fluoride, should not be relied to prevent glycolysis.[7]. Fluoride inhibits enolase, which is far downstream in the glycolytic pathway. Enzymes upstream of enolase remain active and continue to metabolize glucose until substrates are exhausted. The anti glycolytic action of fluoride is delayed for up to 4 hour and has little or no effect on the rate of glycolysis during the first 1–2 hour after blood is collected [8].Therefore the present study aimed to compare the glucose concentrations obtained using different collection tubes (plain vacutainer with no anticoagulants, serum separator tube and sodium fluoride tubes)and also to assess the pattern of change in glucose concentration in the different blood collection tubes at 0 hour , 1 hour , 2 hour , 4 hour , 24 hours after blood collection.

MATERIALS AND METHODS

This cross-sectional study conducted on 50 volunteers recruited from college and hospital of K.S. Hegde Medical Academy after approval from the Institutional ethical clearance committee. Apparently healthy volunteers in the age group of 18- 60 years were included in the study. Patients with Hypertension, diabetes mellitus, obesity, thyroid disorders were excluded. After obtaining the informed consent from each participant, 2ml of blood was collected by antiseptic precautions from anticubital vein in all the three different vacutainers.

Plain and fluoride vacutainers were subjected to centrifugation followed by placement on the analyzer for serum glucose assay. Since the serum separator tube completely separates the serum from the cells after a single centrifugation, it was analyzed directly. Glucose analysis was completed and the values were taken as '0" hour glucose.

Samples were then allowed to stand for "1" hour at room temperature after which they were centrifuged and glucose estimation was done and the values were taken as "1" hour glucose. Samples were then allowed to stand for "2" hour at room temperature after which they were centrifuged and glucose estimation was done and the values were taken as "2" hour glucose. Samples were then allowed to stand for "4" hour at room temperature after which they were centrifuged and glucose estimation was done and the values were taken as "4" hour glucose. All the tubes were then placed in the sample refrigerator at 4^{o} C and then re-assayed at 24 hours. Glucose estimation was done by enzymatic reference method with hexokinase.

Statistical Analysis

Data was represented as Mean and standard deviation of glucose for each of the tubes and also over different time intervals. ANOVA was used to find the significant difference in mean glucose levels of samples collected in different tubes. Post hoc test was conducted for multiple comparisons using SPSS version 20. Repeated ANOVA measurement was used to test for difference in glucose levels between different time intervals of study for each of the vacutainers. Post hoc comparisons was considered for significant ANOVA

results. Karl pearson correlation coefficient test is used to find the correlation of glucose values between the different vacutainers at different time intervals. $p<0.05$ was considered as statistically significant.

RESULTS

Mean glucose concentration in SST and Plain tubes were lesser than that in Sodium Fluoride, but not statistically significant. However, at 24 Hours, significant difference was observed in glucose concentration in sodium fluoride, SST and plain tubes. (Table-1)

There was no significant difference in mean plasma glucose level in sodium fluoride tubes across time periods (Table-2). No significant difference in mean serum glucose level in SST tubes across time periods (Table-3). Significant difference in mean plasma glucose level in plain vacutainer across time periods was observed. Gradual decrease in glucose concentration from observed from 0 Hr to 24 Hr. The 24 hr glucose concentration was significantly decreased when compared to glucose concentration at 0hr, 1 hr, 2 hr and 4 hr (Table-4).

At 0 Hr, glucose values correlated better between Fluoride and SST tubes, followed closely by Plain versus SST tubes (Table-5, Fi-1). At 1 Hr, plain versus SST correlated better (Table-6, Fig-2). At 2 Hr, Fluoride versus SST correlated better (Table-7, Fig-3). At 4 Hr, Fluoride versus SST glucose values correlated better (Table-8, Fig-4). At 24 hrs, Fluoride versus SST glucose values correlated better (Table-9, Fig-5).

Table 1: Comparison of mean glucose values in the three collection tubes at different time intervals

Time interval	Mean ± SD Glucose (mg/dl) (Minimum – Maximum)			p value
	Sodium Fluoride	SST	Plain	
0 Hr	95.70 ± 13.26 (76 – 158)	93.18 ± 13.17 (72 – 156)	93.34 ± 13.08 (73 – 153)	0.565
1 Hr	95.60 ± 13.92 (76 – 159)	92.62 ± 13.59 (64 – 152)	92.56 ± 13.87 (73 – 157)	0.454
2 Hr	94.86 ± 14.54 (77 – 159)	92.68 ± 13.25 (73 – 158)	90.70 ± 14.25 (53 – 158)	0.316
4 Hr	94.96 ± 14.54 (73 – 157)	93.44 ± 15.36 (62 – 156)	89.96 ± 16.60 (55 – 157)	0.259
24 Hr	91.24 ± 14.32 (55 – 154)	92.34 ± 13.32 (69– 156)	71.06 ± 21.88 A**B** (26 – 153)	<0.0001

Note: A – Comparison between plain and sodium fluoride , B – Comparison between Plain and SST, **p value <0.0001

Table 2: Mean glucose values in Sodium fluoride Tubes at different time intervals

Time interval	Mean Glucose level(mg/dl)
0 Hr	95.70 ± 13.26
1 Hr	95.60 ± 13.92
2 Hr	94.86 ± 14.54
4 Hr	94.96 ± 14.54
24 Hr	91.24 ± 14.32
p value	0.476

Table 3: Mean glucose values in SST Tubes at different time intervals

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Time interval	Mean Glucose level(mg/dl)
0 Hr	93.18 ± 13.17
1 Hr	92.62 ± 13.59
2 Hr	92.68 ± 13.25
4 Hr	93.44 ± 15.36
24 Hr	92.34 ± 13.32
p value	0.949

Table 4: Mean glucose values in Plain vacutainers at different time intervals

Time interval	Mean Glucose level(mg/dl)
0 Hr	93.34 ± 13.08
1 Hr	92.56 ± 13.87
2 Hr	90.70 ± 14.25
4 Hr	89.96 ± 16.60
24 Hr	71.06 ± 21.88 $^{A**, B**, C**, D**}$
p value	<0.0001

Note: A: Comparison between 24hrs and 0 Hr, B: Comparison between 24 hrs and 1 hr, C: Comparison between 24 hrs and 2 hr, D: Comparison between 24 hrs and 4 hr, **p <0.0001

Table 5: Correlation of Glucose values in Plain, Sodium Fluoride and SST at 0 hour

Tubes	r value	p value
Plain vs Fluoride	0.979	<0.001
Plain vs SST	0.985	<0.001
Fluoride vs SST	0.987	<0.001

Table 6: Correlation of Glucose values in Plain, Sodium Fluoride and SST at 1 hour

Tubes	r value	p value
Plain vs Fluoride	0.968	<0.001
Plain vs SST	0.974	<0.001
Fluoride vs SST	0.953	<0.001

Table 7: Correlation of Glucose values in Plain, Sodium Fluoride and SST at 2 hour

Tubes	r value	p value
Plain vs Fluoride	0.906	<0.001
Plain vs SST	0.921	<0.001
Fluoride vs SST	0.987	<0.001

Table 8: Correlation of Glucose values in Plain, Sodium Fluoride and SST at 4 hour

Tubes	r value	p value
Plain vs Fluoride	0.845	<0.001
Plain vs SST	0.890	<0.001
Fluoride vs SST	0.948	<0.001

Table 9: Correlation of Glucose values in Plain, Sodium Fluoride and SST at 24 hour

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Tubes	r value	p value
Plain vs Fluoride	0.642	<0.001
Plain vs SST	0.641	<0.001
Fluoride vs SST	0.943	<0.001

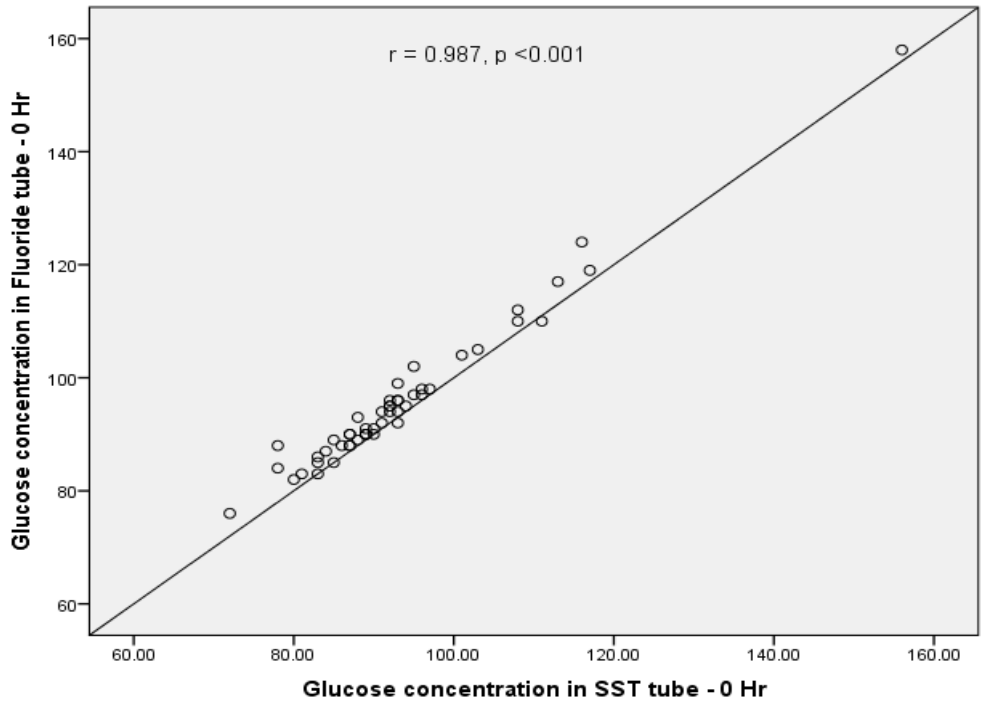

Figure 1: Correlation of glucose values between sodium fluoride and SST tubes at 0 Hour

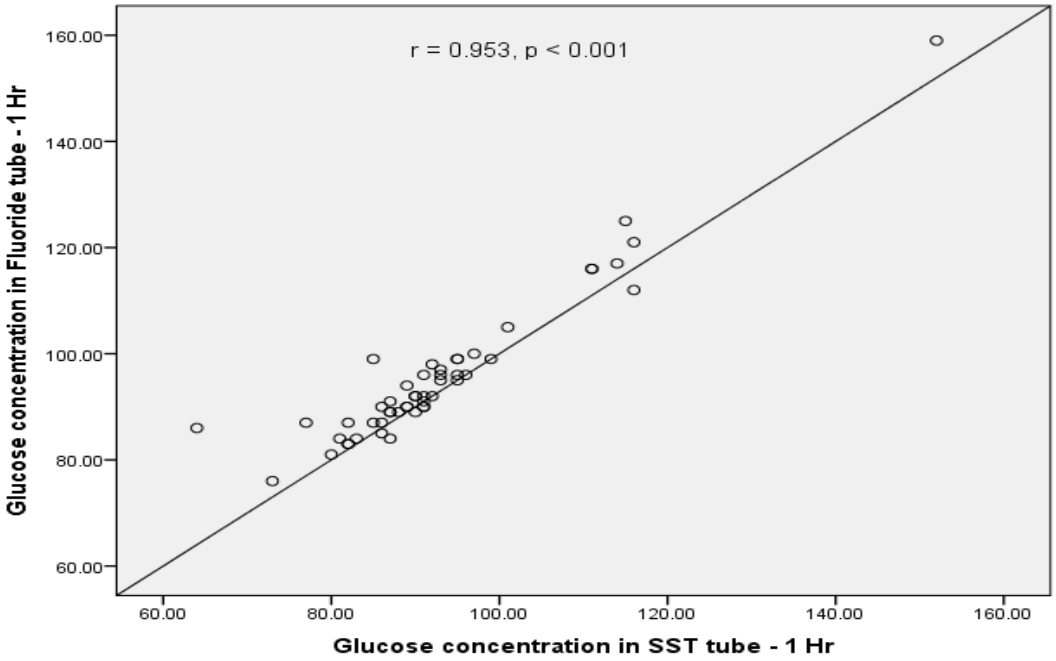

Figure 2: Correlation of glucose values between sodium fluoride and SST tubes at 1 Hour

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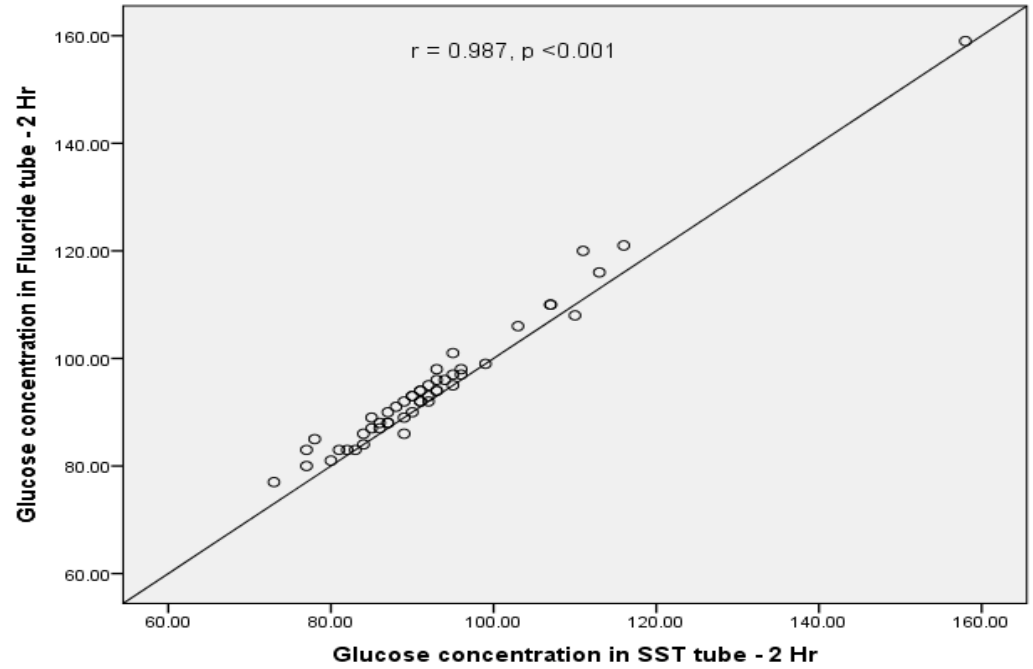

Figure 3: Correlation of glucose values between sodium fluoride and SST tubes at 2 Hour

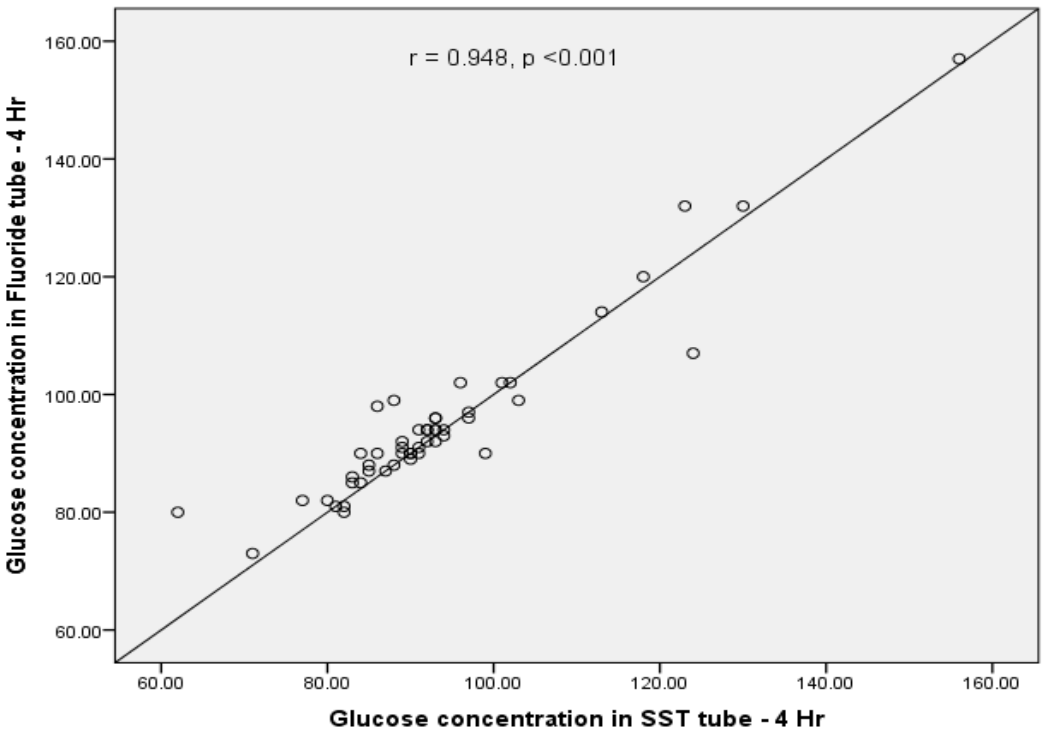

Figure 4: Correlation of glucose values between sodium fluoride and SST tubes at 4 Hour

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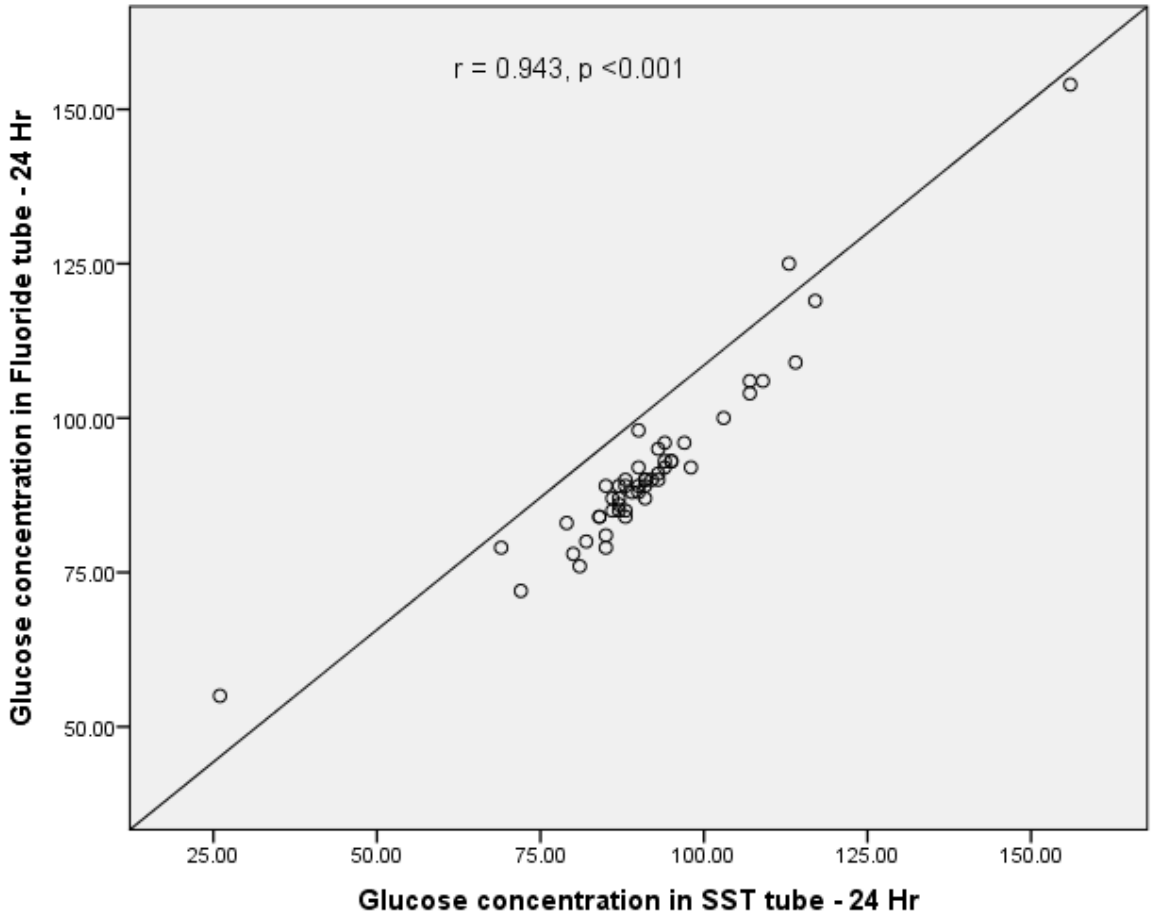

Figure 5: Correlation of glucose values between sodium fluoride and SST tubes at 24 Hour

DISCUSSION

The present study intended to compare glucose concentrations obtained using different collection tubes (plain vacutainer with no anticoagulants, serum separator tube and sodium fluoride tubes) and to assess the pattern of change in glucose concentration with respect to time in the different tubes from the time of receipt in the laboratory and upto 24 hours after collection.

Present study showed that the mean glucose concentration in SST and Plain tubes were lesser than that in Sodium Fluoride, but not statistically significant. However, at 24 Hours, significant difference was observed in glucose concentration in sodium fluoride, SST and plain tubes.

There was no significant difference in mean plasma glucose level in sodium fluoride and SST tubes across time periods. There was a significant difference in mean glucose levels in plain vacutainer across time periods. Present study showed a strong correlation with minimal difference in glucose level between sodium fluoride and SST tubes. ($r = 0.961$, $p < 0.001$)

Al khurasi A et al in his study of comparing plasma glucose in sodium fluoride tubes and serum glucose in SST tubes found an average difference of 0 mg/dl between the two tubes. Study also found a significant correlation ($r= 0.991$) between the two tubes [11].

Fernandez L et al compared blood glucose values in samples collected in SST tubes and sodium fluoride tubes. There was a high rate of hemolysis in the NaF/KOx tubes (86.2%) while hemolysis was infrequently observed with the SST tubes (2%). Comparing only blood draws where no hemolysis was observed in both tubes showed no effect of tube type on serum/plasma glucose concentrations [12].

Present study showed that although there is a significant difference in mean glucose values in SST and sodium fluoride tubes at 0, 1, 2 and 4 hours, it did not exceed 3 mg/dl. Therefore both sodium fluoride and SST tubes can be used to estimate glucose level.

Use of SST tubes can offer many specimen processing advantages as these universal tubes can be used for the majority of biochemistry and immunology tests reducing the need for other consumables and the amount of blood drawn from the patients, and improving turn- around-time and laboratory workflow.

CONCLUSION

There was no significant difference in the mean glucose levels in sodium fluoride and SST tubes across the time periods. The glucose values of sodium fluoride and SST tubes showed a better correlation. Therefore SST tubes can be safely used to estimate glucose levels.

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