Effectiveness of NaF Vials for Estimation of Glucose in NABL Accrediated Laboratory of a Tertiary Care Hospital

Dr. Anita Sharma[1], Dr. A.D Deepak[2*], Dr. Vinita Kalra[3]

[1]Associate Professor, Department of Biochemistry, HIMS, Dehradun, India
[2,3]Professor, Department of Biochemistry, HIMS, Dehradun, India

Abstract: Comparison of glucose level in NaF vacutainer and serum separator vacutainer. The study was conducted at Biochemistry Department of Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand. Total 386 random cases were selected for the study. Glucose levels were measured by the Hexokinase method using a Unicel DXC800 Chemistry analyser (Beckman Coulter India (Pvt.) Ltd.). "Paired t-test" was used for analysis. No significant difference of glucose values were seen amongst the two vacutainers, whereas a significant decrease in glucose levels with time was observed in both the vacutainers statistically. Serum separator vacutainers can be used in place of NaF vacutainers to estimate glucose in the clinical laboratories.
Keywords: NaF vacutainer, Serum Separator Vacutainer (SSV).

INTRODUCTION

The impact of diabetes mellitus in terms of its diagnosis and management by different levels of health care providers has made measurement of glucose among the most commonly requested core biochemistry investigations worldwide both as laboratory-based or point-of-care testing [1]. The blood sugar concentration or blood glucose level is the amount of glucose present in the blood of a human or animal. Normally in mammals, the body maintains the blood glucose level at a reference range between 64.8 and 104.4 mg/dl. The human body naturally tightly regulates blood glucose levels as a part of metabolic homeostasis [2]. The major problem with glucose determinations is the pre-analytical factor of the type of tubes used for blood collection. Since red blood cells metabolize glucose via glycolysis, their presence can lower glucose concentration in whole blood as much as 7 mg/dL per hour [3].

In 1941, sodium fluoride (Na F) containing tubes were introduced into laboratory practice for blood collection for glucose measurement. The use of these tubes appeared to be suitable for blood collection when laboratory practice was not streamlined and there was a long delay in blood separation following collection, a practice that continued, and was not questioned or revisited, for more than five decades [4]. Collection of blood in clot tubes permits the metabolism of glucose in the sample by blood cells until separated. Loss of glucose can be prevented by using fluoride tubes since fluoride inhibits glycolysis [5]. To prevent this occurrence, blood can be collected into red-top vacutainers with a serum separator gel that sequesters the cells from plasma/serum [6].

In the present study, we aimed to compare glucose levels measured in NaF and serum separator vacutainers at different time periods and compare the results statistically. This would give an idea whether SSV can be used in place of NaF vacutainers.

MATERIALS AND METHODS

The study was conducted at the Clinical Biochemistry Department of Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand. Blood samples of total 386 randomized cases were collected in the laboratory in NaF vacutainer and SS vacutainers simultaneously, for measurement of glucose and other biochemical parameters. Sample size was calculated by using the data from previous studies.

Glucose was measured in all samples by the Hexokinase method on Unicel DXC800 Chemistry analyser (Beckman Coulter India (Pvt.) Ltd.). The estimations were done thrice on both the vacutainers. The first estimation was done at 0 hrs immediately after receipt of the samples, second estimation at 6 hours and third estimation at 24 hrs after receipt of the samples.

RESULTS

Descriptive statistics

Group -1 Glucose levels in serum separator vacutainers.

Group -2 Glucose levels in sodium fluoride vacutainers.
Total number of samples N-386

Table-1: Glucose levels (mg/dl) in two groups at the selected different time intervals

Group	N	Mean (mg/dl)	Standard deviation
Group-1 (SS Vacutainers)			
0 hrs	386	138.90	68.903
6 hrs	386	137.17	68.944
24 hrs	386	136.60	68.678
Group-2(NaF Vacutainers)			
0 hrs	386	142.33	69.074
6hrs	386	139.76	68.633
24hrs	386	137.73	68.966

Table 1 depicts the mean glucose levels in the two groups at 0 hrs, 6 hrs and 24 hrs. It shows a fall in glucose level over time was obtained in both the groups. A lesser fall in glucose is reflected in SS vacutainer in comparison to NaF vacutainer, over the selected time intervals.

Table-2: Comparison of glucose levels within groups at the different time intervals

Group	Mean difference	Standard Deviation	Std. Error Mean	p Value
Group-1 (SS Vacutainers)				
Pair-1(0 hrs-6 hrs)	1.736	5.632	0.287	0.000
Pair-2(0hrs-24 hrs)	2.301	5.164	0.263	0.000
Pair-3(6hrs-24hrs)	0.565	5.784	0.294	0.056
Group-2 (NaF Vacutainers)				
Pair-1(0 hrs-6 hrs)	2.567	6.317	0.322	0.000
Pair-2(0hrs-24 hrs)	4.604	7.198	0.366	0.000
Pair-3(6hrs-24hrs)	2.036	8.038	0.409	0.000

A comparison of glucose levels within groups estimated at different time intervals was analyzed using paired sample statistics. A significant decrease was observed in glucose in both the groups over the time intervals selected in the study.

Table-3: Comparison of glucose levels of group 1 with group 2 at different time

Groups(1 and 2)	Mean difference	Std error difference	Significance
0 hrs	-3.425	4.966	0.491
6 hrs	-2.593	4.988	0.603
24 hrs	-1.122	4.955	0.821

As is evident in Table 3, the paired t- test analysis of glucose values in the two groups with time reflects statistically non-significant difference in glucose values among the two groups matched with time.

DISCUSSION

In the present study fall in glucose levels is seen in Na F vacutainers, which has also been reported in a previous study by Gambino, who showed that the commonly used inhibitors of glycolysis are unable to prevent short-term glycolysis. Glycolysis can be attenuated by inhibiting enolase with sodium fluoride (2.5 mg/mL of blood), or less commonly, lithium iodoacetate (0.5 mg/mL of blood). These reagents can be used alone, or more commonly, with such anticoagulants as potassium oxalate, EDTA, citrate, or lithium heparin. Unfortunately, although fluoride helps to maintain long term glucose stability, the rates of decline in the glucose concentration in the first hour after sample collection are virtually identical for tubes with and without fluoride, and glycolysis continues for up to 4 hr in samples containing fluoride. After 4 hr, the concentration of glucose in whole blood in the presence of fluoride remains stable for 72 hours at room temperature [6].

Glucose measurement in plasma or serum can be done without the need for Na F, if blood separation is achieved within reasonable time (less than two hours). Longer delay should be avoided not only for the purpose of prevention of glycolysis but also for maintaining the quality of other analytes including certain electrolytes, enzymes, and hormones that are known to be affected by delay in blood separation. The recent laboratory guidelines of 2011, for diagnosis and management of diabetes mellitus, approved by the American Diabetes Association, no longer recommend

the use of Na F alone to control glycolysis [7]. This recommendation is corroborated by our study. In the present study, no statistically significant variation was observed in glucose values between Na F and SS vacutainers. Similar findings were found in studies done by Fernandez *et al*. and Li *et al*. who found glucose measured in SS vacutainers and heparinized tubes similar to Na F tubes [8, 9]. In our study glucose levels were measured over different time intervals in Na F and SS vacutainers and our results showed less fall in glucose level in SS vacutainer as compared to Na F vacutainer. This reflects that serum separator gel is better in preventing glycolysis by cells, as it effectively separates the serum and cells, thus preventing contact between them.

CONCLUSION

We have found no significant difference of glucose level in NaF and SS vacutainers. The lesser fall of glucose in SSV indicates that stability of glucose level is better in SSV as compared to NaF vacutainers. Multicentric studies are required to confirm the findings. However, based on our study we recommend that NaF vacutainers should be replaced by SS vacutainers for glucose measurement, as it will also help to minimize common preanalytical errors such as contamination, labeling, loading and programming, thereby help to deliver quality results.

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