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EFFECT OF PREANALYTICAL FACTORS ON VARIATION IN GLUCOSE LEVELS

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ABSTRACT

Introduction: Variability in the method of collection affects not only the diagnosis of diabetes mellitus but also monitoring of glucose concentration during treatment of diabetes. This study determines the effect of delay in centrifugation of samples on glucose levels and the stability of plasma and serum glucose over time.

Material and Methods: This study compared the glucose concentrations by two different collection methods. Blood samples were collected in either non -vacuum NaF or heparin tubes in one method and in the second method in either clot activator or serum separator tubes.

Results: The levels of glucose as well as stability of the sample was higher in plasma than serum. Plasma glucose of NaF tubes and serum glucose of serum separator tubes was higher and more stable than plasma glucose of heparin and serum glucose of clot activator tubes.

Conclusion: Samples collected for estimation of glucose should be separated as soon as possible. Monitoring of glucose concentrations should be done by the same collection method.

KEYWORDS : Glucose, Serum, Plasma

Introduction

Glucose is the most common test done in a laboratory accounting for more than one third of the total investigations. This is a test that is affected by a number of factors that include technique of collection, sample type, transport, delay in analysis and type of tube which in turn affects the glucose concentration. Several studies using different set preanalytical variables have given various results. The antiglycolytic action of fluoride was found to be delayed for up to 4 h. [1,2]. Some studies recorded lower glucose concentrations in heparinised plasma were than in serum by 5% [3]. In contrast, other studies found that glucose concentrations are slightly higher in plasma than in serum. [4,5]. Some studies reported that glucose values measured in serum and plasma were essentially the same [6,7].Variability in the use of the type of sample and the time of estimation affects the diagnosis of diabetes mellitus as well as monitoring of glucose concentration during treatment of diabetes. This study was done to determine the effect of delay in centrifugation of samples on glucose levels and to determine the stability of plasma or serum in estimation of glucose levels.

Material and Methods

This was a Cross-sectional study.The subjects were selected by random sampling from the patients attending the central laboratory of the hospital attached to ASRAM medical college, Eluru for investigations. A total 60 samples from either males or females were collected.

Approval by the institutional ethical committee of ASRAM medical college and informed consent from all the subjects enrolled in the study was obtained. The subjects selected in the study were all adults and those who were advised to get blood glucose estimation done in the hospital. The subjects were divided into four groups of 15 each. From each subject either plasma or serum was collected. Plasma was collected in non-vacuum either NaF or heparin tubes, and serum in either clot activator (CA) or serum separator tubes (SST). The temperature of the central laboratory including the collection centre was maintained between 23-24 ºC and humidity at 45%±10.

15 samples were collected in plasma oxalate and serum in clot activator tubes in duplicate, one set was then separated by centrifugation by our laboratory protocol and for the other set centrifugation was delayed by 30 minutes. The second, third and fourth set of 15 samples each were collected in duplicate in plasma NaF and serum clot activator tubes, serum clot activator (CA) and serum separator tubes (SST) and NaF or heparin tubes respectively. The second set of 15 samples each was collected in plasma oxalate and serum in clot activator tubes for comparison. Similarly the third set for comparison of serum glucose in clot activator (CA) and serum separator tubes (SST) and the fourth set in NaF or heparin tubes for comparison of plasma glucose.

As per the protocol of our laboratory, phlebotomists performed the venipuncture in accordance with the established Standard Operating Procedure. All specimens were mixed by gentle inversion at least five times following collection and plasma samples were separated immediately by centrifugation, while serum was allowed to clot for 10-15 min and when coagulation was completed the samples were centrifuged for at least for at least 15 minutes at 3000 g.

Glucose was estimated immediately after separation of plasma or serum which was taken as 0 h (baseline) followed by estimation at 1h, 2h, 6h and 24h. The samples were analyzed by Hexokinase method in Beckman coulter AU 480. Overnight the samples refrigerated the temperature being maintained between 2-8ºC. The samples were not separated into aliquots. Sampling was done each time by taking an aliquot from the primary tube.

Patients in the age group of 20 to 60 years of either sex, both diabetic and healthy patients were included in the study. Haemolysed samples and patients with any haemolytic or coagulation disorders were excluded from the study.

Data was analyzed using SPSS software. The statistical analysis was done by the unpaired two tailed't' test. The statistical significance was kept as a p value of < 0.05.

Results:

Comparison of glucose concentration at baseline levels between NaF and clot activator, serum gel separator and clot activator, NaF and heparin has shown a difference of 2.3%, 3.6% and 2.8% respectively [Table I].Comparison between the plasma and serum glucose by immediate and delayed centrifugation of 30 minutes has shown that the plasma and serum decreased by 1.5 % and 1.9 % respectively when the centrifugation was delayed by half an hour [Table II]. A decrease in glucose concentration was seen in both plasma and serum at 1,2,8 and 24h [Table III A]. Comparison of glucose stability between plasma (NaF) and serum (Clot Activator) has shown a significant difference at 1, 2, 8 and 24hrs. [Table IIIB]. A decrease in glucose concentration was seen in both SGS and clot activator serum at 1, 2, 8 and 24h [Table IVA]. Comparison of glucose stability between SGS and serum (Clot Activator) has shown a significant difference at 1, 2, 8 and 24hrs. [Table IVB]. A decrease in glucose concentration was seen in both NaF and heparin tubes at 1, 2, 8 and 24h [Table VA]. Comparison of glucose stability between the two plasma tubes has shown a significant difference at 1hr, 8hr and 24 hr while no significant difference was noted at 2h [Table VB].

Table I-Comparison in Baseline Glucose

Tubes	%
NaF vs Clot Activator	2.3
SGS vs Clot Activator	3.6
NaF vs Heparin	2.8

Table II-Difference between Plasma and Serum Glucose based on centrifugation time

Tube	Immediate	Delayed	Difference in %
NaF	122.9±34.7	121.1±34	1.5
Clot Activator	120.1±33.9	117.7±33.4	1.9

Data represented as Mean±SD and difference in percentage of glucose by time

Table IIIA-Difference in Glucose stability between Plasma and Serum

Time in hours	NaF	Decrease in glucose %	Clot activator	Decrease in glucose %
0	122.9±34.7	-	120.1 ±33.9	-
1	120.4±34.9	2.0	116.6±33.6	2.9
2	117.1±34.7	4.7	112.9±34	6.0
8	114.9±34.5	6.5	106.9±33.7	11.0
24	111.1±34.7	9.6	100.8±33.7	16.1

Data represented as Mean±SD and difference in percentage of glucose by time

Table IIIB-Comparison of the Glucose stability between Plasma and Serum

Time in hours	Plasma	Clot activator	P value
1	2.7±0.72	3.3±0.62	0.0119
2	5.5±0.99	7±0.84	0.0001
8	8±0.76	13±1.46	0.0001
24	11.8±1.01	19.1±1.50	0.0001

Data represented as Mean ± SD
*p value significant at ≤ 0.05 compared with baseline glucose

Table IVA- Difference in Glucose stability in Serum

Time in hours	SGS	Difference in %	Clot activator	Difference in %
0	155.7±94.3	-	150.1±94.2	-
1	154.9.2±94.5	0.5	145.7±94.5	2.9
2	153.7±94.1	1.3	140.9±93.9	6.1
8	149.5±94.1	3.98	134.1±94	10.7
24	141.7±94.2	9	125.5±83.9	16.3

Data represented as Mean±SD and difference in percentage of glucose by time

Table IVB-Comparison of the Glucose stability between SGS and Clot Activator

Time in hours	SGS	Clot activator	P value
1	0.8±0.67	4.4±1.06	0.0001
2	1.8±1.08	9.4±0.83	0.0001
8	6.2±1.08	16±1.33	0.0001
24	14±0.93	18.3±1.05	0.0001

Data represented as Mean ± SD
*p value significant at ≤ 0.05 compared with baseline glucose

Table VA- Difference in Glucose stability in Plasma

Time in hours	NaF	Difference in %	Heparin	Difference in %
0	198.3±106.6	-	192.8±106.4	-
1	194.4±106.1	1.97	187.9±105.5	2.5
2	189.4±106	4.5	182.4±106.4	5.4
8	185.7±105.7	6.35	177.9±103.9	7.7
24	179.7±105.6	9.4	171.1±104.6	11.2

Data represented as Mean±SD and difference in percentage of glucose by time

Table VB-Comparison of the glucose stability between Plasma NaF and Heparin

Time in hours	NaF	Heparin	P value
1	3.87±1.19	4.87±1.5	0.0527
2	8.87±1.36	9.80±1.7	0.1092
8	12.53±2.03	14.93±3.69	0.0357
24	18.4±3.46	21.73±4.60	0.0347

Data represented as Mean ± SD
*p value significant at ≤ 0.05 compared with baseline glucose

Discussion

The loss of blood glucose due to glycolysis has been studied for several years and several efforts were done to measure the glucose concentration without any loss. Michael Turchiano et al in their study observed that the average glucose reading for the centrifuged tubes containing a clot activator and serum gel separator was significantly higher than the NaF tubes by 0.196 ± 0.159 mmol/L or 4.2%. They used ice to store the samples during transportation and sent to the laboratory within 2-4 hours of being drawn [8]. In contrast, Frank EA et al observed in their study that serum gave values lower than fluoride plasma by 1.15%. On storing the sample at room temperature for 8 hr, the serum glucose value decreased by 8% and fluoride plasma by 4.3% [9]. This study is in agreement with Frank et al that serum value was lower than plasma but by 2.3% at baseline and at 8h the serum value decreased by 10.7-11% and plasma by 6.35 -6.5%.

The mean glucose concentration decreased by 4.6% at 2 h and by 7.0% at 24 h when blood was drawn into tubes containing NaF and sodium oxalate and stored at 37ºC [10] . This study showed a decrease by 4.5-4.7% at 2h and 9.4-9.6% at 24h and the stability of glucose levels were significantly higher in NaF than clot activator tubes at 1,2,8 and 24hrs.

In a study by Li et al, glucose values were the same in the three tubes red, gray, and green even after 4h of standing at room temperature [11].

Dimeski et al observed that in serum the mean % difference from 0h sample was at 3.1%, 5.5% and 10.1% at 1h, 2h and 4h at room temperature [12].

In contrast, in this study the mean % difference from baseline in the clot activator tube was 2.9%, 6.0-6.1%, 10.7-11.0% and 16.1-16.3% at 1h, 2h, 8h and 24h which shows higher % difference at all levels except at 0h.

Al Kharusi in their study observed no significant difference in SST tubes when the samples stored at 4ºC and measured daily for 7 days after 0h sampling [13].

Steele et al observed that at room temperature serum samples centrifuged but left on serum plug were constant for just 2h i.e. 98% of baseline (by 2%) decreasing to87% (by 3%) at 24h [14]. In contrast to both the studies, in this study there was a decrease in glucose concentration in SGS tubes by 1.28 and 9% at 2h and 24 h respectively and the stability of glucose levels were significantly higher in SST than Clot Activator tubes at 1,2,8 and 24hrs.

Sidebottom et al showed that a decrease in glucose was significantly greater in the heparin samples than in the NaF samples at room temperature [2].

According to Chan et al, the decrease of glucose in the NaF tubes paralled that of heparin during the first hour and after 1 hr the rate of decrease in heparin treated samples was much higher than fluoride treated samples at room temperature [1].

Li et al found that the glucose in heparin decreased at 0.3 mmol/L (5.4mg/dl) per day and at 4 h, comparison of glucose concentration between heparin and NaF/KOx tubes was statistically insignificant from one another at room temperature [11]. A study by Dimeski et al showed that at 4 hr glucose concentration in NaKOx decreased by 8.5% decrease, whereas the lithium heparin plasma had significantly higher decrease of 18.5% in glucose concentration at room temperature[12]. Sidebottom et al recorded a decrease in glucose concentration that was significantly greater in the heparin tubes than in the NaF tubes [2] and this study is in agreement with it. In contrast with Dimeski et al in this study the glucose concentration at 8 hr decreased by 6.35%-6.5% in NaF and 7.7% in heparin which is less than at 4 hours and the stability of glucose levels were significantly higher in NaF than heparin tubes at 1,8 and 24hrs.

The differences in glucose concentrations in different studies appears to be due to the potential impact of pre-analytical factors like different sample collection methods, storage conditions and the time of processing . This is as a result of lack of harmonization for glucose assays in the pre-analytical and analytical phases.

Conclusion

Samples that are collected for estimation of glucose should be should be separated as soon as possible. The levels of glucose, as well as stability were higher in plasma than serum. Plasma glucose of NaF tubes and serum glucose of SST tubes was higher and more stable than plasma glucose of heparin and serum glucose of clot activator tubes. Universal guidelines must be adapted to decrease the variability of glucose levels.

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