ORIGINAL RESEARCH PAPER

Biochemistry

A COMPARATIVE STUDY OF BLOOD GLUCOSE WITH OR WITHOUT SODIUM FLUORIDE

KEY WORDS: Glucose,Sodium fluoride, Fluoride vial, Sterile vial

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ABSTRACT

INTRODUCTION: Glycolysis decreases serum glucose by approximately 5-7% in 1 hour (5-10 mg/dl) in normal uncentrifuged coagulated blood at room temperature. Glycolysis has been found to be inhibited and glucose stabilized at room temperature by adding Sodium Fluoride. Fluoride ions prevent glycolysis by inhibiting enolase.
AIM: To determine whether using Sodium fluoride as an anticoagulant really has a stabilizing effect on glucose concentration in blood even after 4 hours of collection.
METHOD: Prospective study of 100 patients. Samples collected with or without sodium fluoride and glucose estimation done after 4 hours of collection.
RESULTS: Glucose level difference between sterile vial sample and fluoride vial sample after 4 hour of storage at room temperature was statistically significant.
CONCLUSION: Fluoride has a very strong stabilizing effect on serum glucose concentration.atleast upto 4 hours storage at room temperature

INTRODUCTION- The Blood Glucose is regulated by the complex interplay of multiple pathways, modulated by a number of hormones. In most clinical laboratories, plasma or serum is used for most glucose determinations. Venous plasma is recommended for diagnosis of Diabetes.[1, 2] Although older methods of analysis reported that glucose concentrations in plasma were 5% lower than in serum[3], a 2004 study indicated that glucose values measured in serum and plasma are essentially the same[4].

Laboratory personnel are constantly faced with the problems of inconsistent power supply such that specimens for analysis are left in the laboratory at ambient temperature for several hours before analysis can be done. Glycolysis decreases serum glucose by approximately 5-7% in 1 hour(5-10 mg/Dl) in normal uncentrifuged coagulated blood at room temperature.[5,6] In separated,nonhemolyzed sterile serum, the glucose concentration is generally stable as long as 8 hours at 25^{0}C and upto 72 hours at 4^{0}C;variable stability is observed with longer storage periods[7] Glycolysis has been found to be inhibited and glucose stabilized for as long as 3 days at room temperature by adding Sodium Fluoride or less commonly ,sodium iodoacetate to the specimen[8] Fluoride ions prevent glycolysis by inhibiting enolase.This inhibition is due to the formation of an ionic complex consisting of Mg^{2+},inorganic phosphate and fluoride ions. Fluoride is also a weak anticoagulant because it binds calcium; however, clotting may occur after several hours. It is therefore advisable to use a combined fluoride-oxalate mixture such as 2mg of potassium oxalate and 2mg sodium fluoride per ml of blood to prevent late clotting. To minimize glycolysis, the cells should be removed within minutes. Alternatively, the tube should be placed in ice water slurry and the cells separated within 30 minutes[2]

AIMS AND OBJECTIVES - To determine whether using Sodium fluoride as an anticoagulant really has a stabilizing effect on glucose concentration in blood even after 4 hours of collection.

MATERIAL AND METHODS- We conducted a prospective study in Department of Biochemistry in Assam Medical College and hospital for 2 months duration from January 2017 to February 2017 and received blood samples of patients from various outpatient departments in our Clinical Biochemistry Laboratory. Total of 100 samples were included for study. We collected samples in empty sterile vials without any anticoagulant as well as in vial containing sodium fluoride. Serum glucose was estimated in both cases when freshly collected as well as after 4 hours .The method used was Hexokinase Method of Glucose estimation by using Siemens Auto analyzer with its dedicated reagents.

HEXOKINASE (GLUC) METHOD:
The GLUC method is an in vitro diagnostic test intended for the quantitative determination of glucose in human serum, plasma, urine, and cerebrospinal fluid. The glucose (GLUC) method is an adaptation of the hexokinase-glucose-6-phosphate dehydrogenase method

Principles of Procedure: Hexokinase (HK) catalyzes the phosphorylation of glucose in the presence of adenosine-5'-triphosphate (ATP) and magnesium to form glucose-6-phosphate (G-6-P) and adenosinediphosphate (ADP). G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) in the presence of nicotinamide adenine dinucleotide (NAD) to produce 6-phosphogluconate and NADH. One mole of NAD is reduced to one mole of NADH for each mole of glucose present. The absorbance due to NADH (and thus the glucose concentration) is determined using a bichromatic (340 and 383 nm) endpoint technique.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HEXOKINASE, Mg2+}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + \text{NAD+} \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADH+H+}$$

The Glucose values were compared between untreated samples and fluoride vial samples.

RESULTS-
On storing the sample at room temperature for 4 hr, the sterile vial sample glucose value decreased by 8%. Even fluoride vial sample had 1.3% lower glucose. This difference between sterile empty vial sample and fluoride vial sample after 4 hour of storage at room temperature was statistically significant at 95%C.I.On sub sample analysis similar results were found in both samples of Diabetic and non Diabetic patients.

SERUM GLUCOSE ESTIMATION
1) STERILE VIAL SAMPLE AT ZERO HOUR AND 4 HOUR LATER
After running Student's Paired T-test, a highly significant difference (at 95% C.I.) was found when comparison was made between Glucose level in sterile vial sample freshly taken and after 4 hours.

	No. OF SAMPLES RUN	MEAN (mg/dL)	SD	STANDARD ERROR
ZERO HOUR	100	133.0	32.1	10.15
4 HOUR	100	102.8	24.5	7.76

TABLE 1: comparison of blood glucose in sterile vial sample

at zero and 4 hour

Student One-Sample T Test

	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
ZERO HR	13.096	99	.01	133.00	110.0268	155.9732
4 HOUR	13.240	99	.01	102.80	85.2359	120.3641

TABLE 2: Student T test analysis of comparison between blood glucose in sterile vial sample at zero and 4 hour

2) FLUORIDE VIAL SAMPLE AT ZERO HOUR AND 4 HOUR LATER

No statistically significant difference was found when blood glucose level was compared between fluoride vial samples at zero hour and 4 hour later i.e Glucose level remains almost same in both the scenario

3) STERILE VIAL SAMPLE AND FLUORIDE VIAL SAMPLE AFTER 4 HOUR

After running Paired-T-test ,a highly significant difference (at 95% confidence interval)was found when comparison was made between Glucose level in sterile vial sample and fluoride vial sample after 4 hours.

One-Sample Statistics

	No. of Samples Run	Mean (mg/dL)	SD	Std. Error Mean
Sterile vial	100	102.8	24.55	7.76431
Fluoride vial	100	131.90	31.66	10.01382

TABLE 3: Comparison of Blood glucose in sterile vial sample and fluoride vial sample at 4 hour

Student One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Sterile vial	13.240	99	.000	102.80000	85.2359	120.3641
Fluoride vial	13.172	99	.000	131.90000	109.2472	154.5528

TABLE 4: Student T-Test analysis of Comparison of Blood glucose in sterile vial sample and fluoride vial sample at 4 hour

DISCUSSION

The present work demonstrates that glucose can be measured reliably in serum samples kept at room temperature if the assay is carried out within 4 hours of specimen collection and serum separation and stored in fluoride vials but this is not true if sterile empty vials are used for sample collection. This is in accordance with a study by Nwosu, O. K. and Nwani, C. D.in which they found that reliable values for serum glucose can be obtained from samples kept at room temperature only if analysis is carried out within 2 hours of sample collection.[9].A study by Nwangwu C.O. Spencer et al showed that Fluoride oxalate had a better stabilizing effect on plasma glucose within the first 30 minutes[10]which is moving in the way of our study that sodium fluoride is stabilizing the blood glucose level even after 4 hours of collection. Milton Weismann et al in their study concluded that fluoride treated samples should be used when a delay of an hour or more is unavoidable[11].Similarly our study also demonstrates that if blood glucose estimation is not possible upto 4 hours of collection, then fluoride vials are best suited for the purpose.

CONCLUSION

Fluoride has a very strong stabilizing effect on serum glucose concentration. Using Sodium fluoride in blood glucose estimation makes our results reliable even if there was a delay in processing of sample for upto 4 hours after collection.

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