Original Research Article

Determining the efficacy of citrate buffer tubes compared to Sodium fluoride tubes as inhibitor of glycolysis in glucose estimation

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A B S T R A C T

Ex vivo glycolysis is the main interference factor in glucose estimation. In the unpreserved blood sample the glucose concentration decreases rapidly, because of glycolysis. Sodium fluoride, inhibits enzyme Enolase of glycolysis pathway. The latest guidelines provided by American Association for clinical chemistry (AACC) and American Diabetes Association (ADA) for laboratory analysis have recommended the use of tubes containing citrate buffer as inhibitor of glycolysis. It inhibits hexokinase and phosphofructokinase.
Aims: To determine the efficacy of citrate buffer tubes compared to Sodium fluoride tubes as inhibitor of glycolysis in glucose estimation.
Materials and Methods: 110 blood samples collected for Glucose estimation were included in the study. From 5ml of collected blood one ml each was added in pairs of sodium fluoride and citrate buffer tubes, labelled as 0 and 2hrs respectively. Glucose estimation was done on ERBA CHEM 5 V2 Semi-automated analyzer by Glucose oxidase -peroxidase (GOD-POD) kit method.
Results: The analyzed levels of glucose obtained in NaF and citrate buffer at 0hrs was 110.07±36.72mg/dl and 111.10±36.95mg/dl and at 2hrs was 104.00±36.20mg/dl and 105.90±36.86mg/dl. Statistically significant difference was observed between the glucose levels obtained from both the tubes. The difference between mean values of glucose concentration at 0hr and 2hrs for sodium fluoride and citrate buffer tubes was 6.07±3.06mg/dl and 5.20±2.43mg/dl respectively.
Conclusion: The study concludes that there were statistically significant higher levels of glucose in citrate tubes compared to fluoride tubes, which can help to reduce the preanalytical errors in glucose estimation due to glycolysis. There is a need for further studies with improved composition of citrate buffer and studies to evaluate the clinical impact of difference in glucose levels of both tubes in diagnosing diabetes.

1. Introduction

Plasma glucose levels play an important role in the diagnosis and monitoring of diabetes mellitus.[1] However glycolysis ex-vivo is the main interference factor in glucose estimation.[2] Various inhibitors are being used as inhibitors of ex vivo glycolysis to stabilize glucose levels in the collected blood samples.[3,4] The most frequently used inhibitor is sodium fluoride (NaF).[5] Enolase an enzyme of glycolytic pathway is inhibited by Fluoride. But the drawback of using sodium fluoride is that, the enzymes present above enolase are active and they keep metabolizing glucose to produce the respective substrates.[6] The antiglycolytic action of fluoride is delayed up to 4hours and has little or no effect on the rate of glycolysis during initial 1-2h after blood is collected. The rate of glycolysis is known to decrease the levels by 5-7% per hour (approximately 0.6mmol/L (10mg/dl))[5] – This decrease depends on glucose concentration, temperature, leukocyte count and other factors.[7] The decrease in glucose concentration may cause the diagnosis of diabetes to be missed in the individuals who have glucose levels near the borderline.[8]

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The guidelines of "American Association for clinical chemistry (AACC) and American Diabetes Association (ADA)" in 2002 were modified in 2011, according to it- The tubes in which the sample is collected for Glucose estimat ion should be immediately kept in ice water slurry and plasma should be separated within 30 mins. If that cannot be done, citrate tubes should be used for sample collection.[8]

Citrate inhibits the enzymes hexokinase & phosphofruc-tokinase which act early in the glycolysis pathway. In blood cells, inhibition of glycolysis takes place when the blood pH is in between 5.3 to 5.8. The inhibitory effect of citrate can be maintained for about 8-10hr at 23-25°C.[7] To maintain the inhibitory effect for a long time a pinch of NaF was added and to inhibit enzyme activity by chelating magnesium, EDTA was added.[4]

2. Aim and Objective of the Study

To determine the efficacy of citrate buffer tubes compared to Sodium fluoride tubes as inhibitor of glycolysis in glucose estimation.

3. Materials and Methods

3.1. Procedure for preparation of tubes

1. Preparation of Citrate buffer tube :

Citric acid: Sodium citrate: Disodium EDTA: Sodium fluoride, in the ratio of 3.5:1.6:4.8:0.2.
Each collection tube contains 10mg/ml of this buffer.[7]

1. Preparation of Sodium fluoride tube:

Potassium oxalate: Sodium fluoride in the ratio of 3:1
Each collection tube contains 2mg/ml of it.[7]

110 fresh blood samples collected for Glucose estimation at Dr Prabhakar Kore Charitable Hospital, Belagavi were included in the study. Permission to conduct the study was obtained from Institutional Ethics Committee on human subject's research of Jawaharlal Nehru medical college, Belagavi. Only Fresh samples obtained for blood glucose estimation were included and Samples collected before an hour and Hemolysed samples were excluded. From 5ml of collected blood one ml each was added in pairs of sodium fluoride and citrate buffer tubes, labelled as 0 and 2hrs respectively. One tube each of sodium fluoride and citrate were centrifuged and analyzed immediately considered as 0 hour and second tubes of each were centrifuged and analyzed after 2 hours. During those 2 hours the samples were kept at room temperature of around 22–25°C. The glucose levels were estimated in the ERBA CHEM 5 V2 Semi-automated analyzer by Glucose oxidase-peroxidase (GOD-POD) kit method.

4. Results

The glucose levels measured in citrate buffer and sodium fluoride tubes at 0hr and 2hrs are given in [Table 1]. The difference in glucose levels between sodium fluoride and citrate buffer tube ranged from -1.03 mg/dl at 0hr to -1.90 mg/dl at 2hr. Glucose levels decreased significantly in both citrate and sodium fluoride tubes [Table 2]. Sodium fluoride and citrate buffer comparison at 0hr to 2hrs was done by applying dependent t-test in which Mean±SD for 0hour for sodium fluoride was 6.07±3.06 and for Citrate buffer it was 5.20±2.43 and p-value was 0.0046 which was statistically significant [Table 3].

Table 1: Mean of glucose values at the different time interval

Sample at the different time interval	Mean±SD
Citrate- 0hr	111.10±36.95
NaF- 0hr	110.07±36.72
Citrate- 2hr	105.90±36.86
NaF- 2hr	104.00±36.20

Citrate: Citrate buffer, NaF : Sodium fluoride, hr: Hours, SD: standard deviation.

Table 2: The difference in glucose levels between citrate buffer and sodium fluoride tubes at the different time interval

Pairs (n=110)	Means of differences (mg/dl)	P - value
Citrate 0h - Citrate 2h	5.20	0.0001
Citrate 0h - NaF 0h	-1.03	0.0001
NaF 0h - NaF 2h	6.07	0.0001
Citrate 2h - NaF 2h	-1.90	0.0001

Citrate: Citrate buffer, NaF : Sodium fluoride, hr: Hours.

4.1. Correlation between Sodium fluoride and Citrate buffer at 0 hours and 2 hours time points by Karl Pearson's correlation coefficient method

The correlation between Sodium fluoride and Citrate buffer was done by Karl Pearson's correlation method, Sodium fluoride and Citrate buffer at 0 hours and 2 hours showed strong correlation. The r- value for 0 hours was (0.9944) and the p-value was p<0.05 which was statistically significant. Similarly the r-value for 2hours was 0.9919.the p-value was p<0.05 which was statistically significant [Table 4].

5. Discussion

Glucose estimation plays a key role in the diagnosis and management of Diabetes Mellitus. Previous studies have shown that sodium fluoride tubes show lower levels of glucose as compared to citrate buffer tubes.[9,10] This is because of ineffective inhibition of glycolysis by sodium fluoride tubes during the gap between blood sample collection and its analysis. It is known that errors in

Table 3: Comparison of change from 0 hours to 2 hours Sodium fluoride and Citrate buffer by dependent t test

Variables	Mean	Std.Dv.	Mean Diff.	SD Diff.	% of change	Paired t	p-value
Sodium fluoride	6.07	3.06					
Citrate buffer	5.20	2.43	0.87	3.16	14.36	2.8901	0.0046*

*p<0.05

Table 4:

Time points	Correlation between Sodium fluoride and Citrate buffer scores at			
	r-value	r^2	t-value	p-value
0 hours	0.9944	0.9888	97.5970	0.0001*
2 hours	0.9919	0.9838	80.9480	0.0001*
Changes	0.3545	0.1257	3.9404	0.0001*

*p<0.05

estimation of blood glucose levels will increase the chances of missing the diagnosis of Diabetes Mellitus. This needs the replacement of sodium fluoride tubes with an effective inhibitor of glycolysis like citrate buffer as recommended by American Association for clinical chemistry (AACC) and American Diabetes Association (ADA) in 2011. The presently accepted guidelines for glucose values in diagnosis of diabetes are according to the data taken from glucose estimation done using sodium fluoride tubes, this also needs to be changed.[11]

Previously a study was done by Gupta et al[3] in which a comparison was done between the glucose levels estimated from citrate buffer tubes and sodium fluoride tubes. They concluded that the glucose levels were lower in sodium fluoride tubes compared to citrate buffer tubes. Our study has also given similar results the mean difference of glucose values between sodium fluoride tubes of 0hr and 2hrs was 6.07 and the mean difference of glucose values between citrate buffer tubes of 0 hrs and 2hrs was 5.20, which implies significantly lower values of glucose in sodium fluoride tubes as compared with citrate tubes.

Another study was done by del Pino et al[12] in phases, in the beginning they validated the use of citrate buffer tubes for estimation of glucose, then they compared the values of glucose in both sodium citrate & fluoride tubes. They found significant difference in the glucose levels estimated from both the tubes. In our study the difference in mean values ranged from -1.03 mg/dl at 0 hrs to -1.90 mg/dl at 2 hrs in citrate buffer tubes & sodium fluoride tubes, and the p value was found to be less than 0.0001, which showed a statistically significant difference in glucose results in these two types of collection tubes at 0 hrs and 2 hrs.

Another study was done by Toro crespo et al[11,13] where glucose levels were analyzed in citrate buffer tubes and sodium fluoride tubes and they found the 0hr mean values between citrate buffer and sodium fluoride tubes had difference of 10.87 in the glucose values, and 2hrs mean values between citrate buffer and sodium fluoride tubes had difference of 14.79 and the observed p value was less than 0.001, which implies significant difference in the glucose values obtained from both tubes. In our study the difference in mean values range from -1.03 mg/dl at 0 hrs to -1.90 mg/dl at 2 hrs in citrate buffer and sodium fluoride tubes, the p value was less than 0.0001, which is statistically significant, though the observed p<0.001 considered to be statistically significant but the mean difference in the values of both the tubes was as low as -1.03 and -1.90 compared to the above study whose mean difference was 10.87 and 14.79.

We also compared the mean difference in values analyzed at 0hrs and 2hrs in NaF with the mean difference in values of 0hrs and 2hrs in citrate buffer tubes, it was found to be 0.87 which was statistically significant.

We observed that there was significant difference in the levels of glucose, analyzed by sodium fluoride tubes and citrate buffer tubes when compared by dependent t test, but Karl Pearson correlation between the values was almost near to 1 which shows the values were almost similar.

6. Conclusion

Glucose, being the most frequently done analysis in the biochemistry laboratories should be given accurate results for proper diagnosis of diabetes which helps in better decisions of patient care.

The study concludes that there were statistically significant higher levels of glucose in citrate tubes compared to fluoride tubes, which can help to reduce the preanalytical errors in glucose estimation due to glycolysis but there was fall in the levels of glucose after 2hr estimation in both the tubes which indicates the need for further studies with improved and standardised citrate buffer tubes. Further studies to evaluate the clinical impact of estimated glucose levels in both tubes for diagnosing diabetes can be taken up.

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9. Conflict of interest

No conflicts of interest

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